

15 July 1982

No room on the space shuttle?

The United States seems bent on giving the shuttle programme a more military emphasis. The outcome could be an expensive but fruitless race for the anti-satellite satellite.

Mr Buck Rogers, the strip-cartoon hero who captains a spacecraft in a cowboy hat, is in danger of being overtaken by reality. Has he come to rest in the Reagan White House? That at least is one explanation of what the United States Administration has been saying about the military uses of the shuttle, as reported elsewhere in this issue. Yet in another sense, there is little new in what the Administration is now saying. Neither the Department of Defense in Washington nor the National Aeronautics and Space Administration has concealed the intention that the shuttle should be used for military purposes, research and development to begin with, the launching of operational military satellites when the techniques have been developed. In part, what has happened now is that the once-distant horizon has come closer. But there have also been technical problems with the shuttle, the increased turn-round time in particular, that will ensure that the machine will spend a much greater proportion of its time than planned working for the military even to meet the original objectives. But the ambitions of the military have also grown since the shuttle was first planned. So are wars in space now at hand? And what kinds of wars are they likely to be? And how can there be wars in space of any kind, when both the competent powers concerned, the Soviet Union and the United States, have been signatories for the past fifteen years of a treaty defining the principles on which "outer space" may be explored and used?

The explicit goal linked with last week's announcement in Washington is that of developing an anti-satellite system, a device for putting paid to other people's satellites. In the Pentagon's view, the objective must seem entirely legitimate, for the Soviet Union tested such a device as long ago as 1967. By that time, the Pentagon had already built its own anti-satellite system based at Johnston Island in the Pacific, but both the superpowers have hitherto been planning anti-satellite systems based on the use of explosives (conventional or nuclear) whose effectiveness in the vacuum of outer space is debatable. So the challenge, as it must now seem, is to make use of newer technologies, those of lasers in particular, to provide more certain means of putting other people's satellites out of action. At the same time, the quest for better methods of surveillance of what is happening on the surface of the Earth continues unabated, while the usefulness of satellites for helping military machines to navigate towards their targets is only now beginning to be exploited. The Stockholm International Peace Research Institute has just compiled a fascinating compendium* of data on the present military uses of outer space that should give pause to anybody who thinks that everything in orbit is by definition peaceful, or that, in this world of fantasy, nothing more dangerous than science fiction can happen. For the treaty that the superpowers have signed goes no further than to require that they should not put "nuclear weapons or any other kinds of weapons of mass destruction" in orbits about the Earth. And a satellite that can knock down other satellites is not a weapon of mass destruction.

The vacuum of outer space is thus a legal as well as a physical vacuum. When the first Earth satellites were launched a quarter of a century ago, there were vigorous debates among academic lawyers about the rights of one state to launch satellites that for mechanical reasons were bound to violate without permission the

"air space" of other states, usually defined as an irregular conical volume with its apex at the centre of the Earth and intersecting national boundaries. In reality, no state has protested at the frequent and unauthorized infringements of this old-fashioned principle, whose demise is in any case sanctified by the treaty on outer space which, without saying precisely where outer space begins, gives that region something of the status of the high seas. (Those who launch satellites continue to be their owners wherever they may be and also, of course, responsible for whatever they may do.) Nothing in the treaty says what should happen to military satellites, say those designed for military surveillance, for the spirit of the treaty is that outer space should be entirely pacific. The practice of recent years shows that it is not. The Soviet Union is the more prolific launcher of reconnaissance satellites, but the United States appears to make better use of its expenditure on this hardware.

So what will happen if and when one or other of these two powers decides that the time has come to forsake experiments in which some expensive satellite is launched into an orbit and then put out of action by an even more expensive anti-satellite satellite, and decides to practise on the other fellow's? Or if one party or the other, engaged in some local conflict the outcome of which might be crucially determined by orbital reconnaissance, decides that it cannot condone the free flight of hostile satellites, however passive their military function? The temptation to put them out of action would be strong, and would be restrained principally by the certainty of retaliation and by the knowledge that, as time goes on, satellite navigational systems will be indispensable parts of strategic missile systems. In other words, anti-satellite satellites are yet another means by which small conflicts can escalate into nuclear conflicts. Yet, as things are, this novel technology seems destined to remain outside the scope of the strategic arms control negotiations under way at Geneva until it is full-blown and, therefore, is more difficult to control.

The circumstances are exactly parallel to the development of anti-ballistic missile systems, which were feared in the 1960s not merely because of their potentially high cost but because they threatened to make unstable the then existing strategic balance. The consequence of that fear was the treaty now called Salt 1, in due course ratified by the United States Senate, limiting the deployment of anti-ballistic missile missiles. It seems not to be appreciated that the costs of the race to perfect anti-satellite systems, and then of making existing reconnaissance satellites less vulnerable, could be just as great — and the results just as uncertain. The best course would be that the two powers concerned should say more openly what they are up to in outer space — and then agree to stop.

In the aftermath of the predictably disappointing end of the United Nations special assembly on disarmament, even the chance that there may be a bilateral agreement between the superpowers on anti-satellite systems will not seem sufficient. What if, one day, there are other powers able to launch reconnaissance and navigation satellites, and other satellites to knock them down? Three-cornered conflicts in which the injured party would not know who did the damage would be more hair-raising than those now in prospect. The only way out of that conundrum is that the passive military uses of space, the capabilities of the satellites concerned and even the data that they

*Outer Space — A New Dimension of the Arms Race. Edited by Bhupendra Jasani. (Taylor & Francis Ltd for SIPRI, 1982, £18.50.)



gather should be registered with some central but impartial body. Such a paradigm for arms control, distasteful though it would be to United Nations professionals, would serve well even in conventional near-wars. Who will give that a try?

Over-anxious politicians

A House of Commons committee complains that biotechnology has been neglected

For the past three years, British politicians have been unusually excited about an emerging technology, biotechnology as it is known. Now, rumour has it, the House of Commons Select Committee on Education and Science is about to castigate the British government for failing to do its duty and to provide sufficient encouragement for this new way of making protein materials with commercial value. The argument is that unless the British government does something from its central position and with its taxpayers' money, a golden opportunity will be lost forever. It is to be hoped that the committee (whose report is not yet published) will have a chance to think again before it puts its reputation behind this dubious proposition.

Ever since the Asilomar conference called in 1975 to argue out the hazards of recombinant DNA research, it has been plain that the new techniques would have great commercial potential. With the recognition that the hypothetical risks of the research are less than at first supposed, there has been a torrent of attempts to make profits from the new techniques. In many ways this new industry differs from others in the past, the development of the steam engine for example, in not seeming on the face of things to be capital-intensive: a few micrograms of restriction enzyme in the hands of a talented person might mean a new product almost overnight. This is no doubt the reason why the past few years have also seen the mushrooming of corporations built around a few talented people with venture capital from some commercial source and dedicated to the notion that it is possible to turn ideas into gold. By comparison with what has happened elsewhere, however, new companies of this kind have been almost conspicuous by their absence in the United Kingdom.

Trendily, politicians and others prefer not to be left out of such excitement. In Britain, especially, it is always possible to raise a wave of patriotism with a simple calculation of how much foreign currency would have been earned in the 1950s if only penicillin had been patented when it had been identified. This is spirit in which a distinguished committee under the late Sir Alfred Spinks was convened three years ago. That committee's report, in April 1980, was, however, more level-headed, acknowledging that university research in molecular biology could with advantage be spread outside the centres in which it was already strong, and that a modest public investment in a few commercial enterprises would probably be worthwhile. Since then, the University Grants Committee has made an extra £800,000 available in earmarked grants to universities (called "chicken-feed" by some) while the National Research Development Corporation (now the British Technology Group) has invested £5 million in the company called Celltech.

The politicians, however, seem still to be dissatisfied. There are several reasons why they should contain themselves in patience. First, the long-term potential of biotechnology is beyond dispute but, as the experience of newly established corporations has recently shown, discovery may be easy but development is likely to be painfully slow (and expensive). Second, there is no strong reason to suppose that established companies are failing to take up the challenge of the new techniques even if their commercial interests dictate silence rather than the opposite. Third, even if a rash of new companies is a sign of health and a guarantee of successful exploitation, the fact that such a rash has not happened spontaneously is more significant than anything the government could do centrally to redress the balance. Instead of complaining that biotechnology is being neglected in Britain, the politicians would better advised to understand why innovation as such is too much neglected and then to correct that failure.

Sinking Law of the Sea

The United States says it will not sign the treaty on the Law of the Sea. It should have said so earlier.

The Law of the Sea is in trouble again. After eight years of negotiation, the United States Administration has now said that it will not sign the draft treaty produced in New York last month. The issue that has stuck in the Administration's throat is predictably that of sea-bed mining, the rules under which the exploitation of metalliferous nodules on the sea-bed will in future be permitted. The circumstances that have now arisen thus resemble those after the First World War, when the United States took a leading part in negotiating the constitution of the League of Nations, the predecessor of the United Nations, but then laconically declared that it would not itself be joining. There is also, however, a parallel between the present Administration's distaste for the Law of the Sea treaty and its pre-election repudiation of President Carter's second instalment of the Salt treaties, negotiated with the Soviet Union and signed but never put to the test of ratification in the United States Senate. The ideological change in Washington at the beginning of 1981 was plainly every bit as sharp as had been advertised in advance.

The delay is above all a shocking illustration of the Reagan Administration's way of conducting international business. The shabby tail is all too well known. For the past several years, grumbling by commercial mining companies in the United States has been growing about the draft proposals in the draft treaty concerning the exploitation of minerals beyond the 200-mile limits in which nation states have an overriding economic interest. The grumbles have not been mere selfishness, but have included many cogent objections to the proposed regime. How can a mining company embarking on the exploitation of a particular patch of sea-bed promise to find an "equivalent" patch to be exploited for the benefit of developing countries? And how is it then to arrange the obligatory "transfer of technology"? If the Reagan Administration had said at the beginning that these proposals were unrealistic, there would no doubt have been a row, but its right to say so could not have been challenged. The Administration will not be as easily forgiven for having taken part in the final negotiating session in New York this year without having said what it now claims it had meant all along to say — that the proposals on sea-bed mining were unacceptable.

What happens next is in the lap of the gods. The ceremony at which the treaty will be signed has already been arranged at Caracas in December (but the government of Venezuela voted against the draft treaty on the grounds that it does not resolve the long-standing dispute with Colombia about maritime rights). Between now and then, the draft treaty can be amended by correspondence. So why not accommodate the United States by excising from the treaty the proposals that give offence, leaving the whole question of deep-ocean mining to some future conference? Unfortunately, the draft treaty on the Law of the Sea, like other international treaties, is a package that cannot easily be unwrapped. Indeed, the proposals on sea-bed mining have so far been a political cement by which maritime and developed countries of the world have traded the right to sail unimpeded through the Straits of Malacca (between Malaysia and Sumatra, part of Indonesia) and similar stretches of water for an understanding that they will return benefits in kind, some sharing of the hypothetical wealth scattered on the deep ocean floor. The notion that such a promise could engender agreement about the international use of the high seas was probably mistaken even when it was first raised more than a decade ago; now that the technology is more nearly usable, it is altogether too contentious for anybody's comfort. The best course now would be that the United Nations should bite the unpalatable bullet with which it has been presented, postpone the signing ceremony and reconvene the conference. But even that course will not be possible unless the United States can promise that it would say what is on its mind. On recent form, that is improbable.

Pentagon stakes claim on shuttle

Military space use forecast by new directive

Washington

The growing unease over the military's role in the US space programme was hardly calmed last week by a new presidential directive on space policy. Although the directive does not actually establish any new programmes, and consists for the most part of vague generalities, it is the most overt and explicit statement of US military space objectives ever issued by a president, and appears to reverse a long tradition of playing down the military side of American space endeavours.

And while officials of the National Aeronautics and Space Administration (NASA) are taking encouragement from the presidential fanfare that accompanied the directive — including a speech at the 4th of July shuttle landing in which President Reagan extolled the benefits that the space programme has reaped for American jobs, technology and spirit — they clearly would have been much happier had the President offered a firm commitment to future projects in the civilian space programme. NASA had in particular hoped for a go-ahead on a fifth shuttle orbiter and what it hopes will be its next big project, an orbiting space station.

The secret military payload on the recent shuttle flight (which is generally known to have contained the Air Force's CIRRIS infrared sensor, being developed to spot missile launches) was a visible reminder of what all the concern was about. Critics say that the shuttle, with its dual role, has effectively erased the line between civilian and military space missions. And they see NASA's acceptance of a substantial Department of Defense (DoD) role, first in the design specifications and now in the operations of the shuttle, as having been a Faustian bargain: NASA received support for a big engineering project of the sort it thrives on, but is now having to pay the price of sacrificing its civilian role more and more to military interests.

These worries have reached even strong supporters of military space projects. Senator Harrison Schmitt (Republican, New Mexico), a former astronaut, is for example seeking to redress the balance between civilian and military space programmes. The Senate's Commerce, Science and Transportation Committee earlier this year approved his proposal to make DoD increase its contributions to the shuttle by \$409 million. Schmitt says this is nothing more than a requirement that DoD should pay for use of the shuttle at the same

rate as all other users. Under Schmitt's plan, NASA would use this extra money to build a fifth shuttle and to increase its research programmes in space science and aeronautics.

The President's science adviser, Dr George Keyworth, argues that this concern is misplaced. In a briefing for reporters on the new space policy, he pointed out that the military goals set out in the directive — most notably the "development of an anti-satellite capability . . . to deny any adversary the use of spaced-based systems that provide support to hostile military forces" — are nothing new. But he was unwilling to shed any light on why the military space programme was being made so explicit for the first time, or why an inter-agency committee set up to implement space policy under the directive will be headed by the White House's National Security Advisor.

While Keyworth spoke of the Administration's commitment to NASA's scientific mission and its general willingness to maintain NASA's current level of funding, he offered little encouragement for supporters of another big civilian project for NASA. He said a reference in the President's 4th of July speech to "establishing a more permanent presence in space" was definitely not a go-ahead for NASA's space station plans. Although "nothing is excluded", he said, "I have not yet seen comprehensive, well thought out plans for what it [a space station] will do."

Keyworth also took issue with Schmitt's plan to raise DoD contributions. NASA's budget, he said, took into account the military missions that NASA will launch; if

NASA receives an additional \$409 million from the military, then its budget should be cut by an equal amount.

Development of military hardware remains solely in the hands of the military. The new policy does not change that; nor does it affect the substance of those projects, beyond guaranteeing the Administration's commitment to them.

According to defence analysts, Air Force publications and published budget data, these projects range from surveillance efforts — such as the CIRRIS infrared telescope tested on the recent shuttle flight — and other "passive" missions such as navigation and communications to "active" anti-satellite capabilities. Anti-satellite work makes up the bulk of research and development in the military space programme; emphasis now is on the development and testing within the next few years of an anti-satellite "miniature homing vehicle" designed to be launched from an F-15 fighter. This weapon disables a satellite simply by ramming it. According to defence analysts, it is not likely to be able to reach targets above 1,000 km.

Basic research and development on lasers and particle beams, with the ultimate aim of producing a more sophisticated anti-satellite weapon and possibly a space-based anti-ballistic missile system, is also receiving a lot of attention.

Another major project is the NAVSTAR "Global Positioning System", a package of 18 satellites that will allow a person on the ground equipped with a receiver to determine his position. Receivers could also be installed in missiles.

NASA's involvement in any of these

Severe cuts unsettle Belgian universities

Brussels

Belgium's universities are still reeling from the unexpected severity of the cuts in public subsidies announced by the government last month. One thousand million Belgian francs (£11.7 million) are to be pared from the resources of the already struggling universities.

The centre-right government hopes to save BF8,000 million in the whole education budget as one of many measures intended to reduce its considerable budget deficit. Savings of BF500 million will be achieved by reducing by 25 per cent the subsidies per student and by widespread salary cuts. The universities are expected to pay for teaching, administration and research out of the allocations per student even though they have already made economies.

But the government wants more high-technology research to be carried out for which reason the research and teaching functions in the universities may have to be separated; a move has already been made in this direction with the allocation of the first

tranche of BF500 million for a programme of industrial research and development. It is hoped that the programme will help to win more public supply contracts in such fields as aeronautics, information technology and telecommunications. Hitherto, most of these contracts were given to research centres or companies.

Meanwhile, some Belgian universities are trying to make money from their own research. The Université Libre de Bruxelles, whose annual budget has been cut by 10 per cent, has started up a special unit to help seek outside finance and markets for the university's research.

The cutbacks will also lead to a shake-up among Belgian academics. Professors will retire at 65 instead of 70 as at present, newly employed graduate researchers will be paid less than they are now and promotion will come more slowly and less frequently. The remaining professors may have to work harder, with inter-university chairs being created to allow experts to share their knowledge more widely. **Jasper Becker**

military projects will certainly continue to be limited to getting them up. The chief fear of supporters of the civilian space programme is that with the increased emphasis on these military uses of space, NASA may end up doing little more than running a bus service, and may, as suggested in *Space World* recently, become in effect a "vassal" of the Air Force. The announcement of an 85 per cent rise in the rates charged to civilian users of the shuttle for flights after October 1985 and a scheduled tripling of charges for LANDSAT data this October — not to mention uncertainty over the government's continued support for LANDSAT — have added doubts about the Administration's commitment to the civilian space programme.

Stephen Budiansky

Franco-Japanese collaboration

Plumbing depths

France is flirting with Japan. Tsukuba, the Japanese "science city" outside Tokyo, is to be named as a twin with Orsay, the complex of scientific institutions and industry south of Paris. At the end of this month, officials from the French ministry of research and technology will be visiting Japan to study Japanese management of science and technology.

M. Jean-Pierre Chevènement, the minister of research and industry has already visited Tokyo twice in his year as a minister. Will he try to model the new ministry of research and industry on its Japanese counterparts?

Philosophical uncertainties apart, one concrete new Franco-Japanese project has been set up — JASP, an oceanographic study of the Japan trench. The objective is scientific, but since the trench involves the subduction zone that causes Japan's frequent earthquakes, there is practical interest in the outcome.

Japan also stands to gain a knowledge of French submersible technology in the form of "SM 97". This new exploration submersible capable of reaching a depth of 6,000 metres is being built at the French oceanographic research institute at Brest, and may be ready by 1985.

The research will take place in two stages, the first in 1984, when the surface research vessel *Jean Charcot* will use a narrow-beam echo sounder (called Sea Beam) to draw a profile of the trench on a scale 1/10,000 to 1/20,000, while Japanese groups make gravimetric, magnetic and seismic measurements. Special study will be made at points where sea-mounts are being subducted and at the point of intersection of the Pacific, Philippine and Eurasian plates.

In the second stage, in 1985 SM97 will be sent down to points reckoned from the first survey to be of particular interest. Three transmitting seismographs and an inclinometer will be left behind.

Robert Walgate

India to expand space technology

Lucknow

India's determination to achieve self-reliance in space technology is evident from the government's recent approval of a space programme for the rest of the decade to build one satellite and two launch vehicles at a cost of 3,963 million rupees (£240 million). An 800-kilogramme remote sensing satellite will be used from 1985 to provide resource information in agriculture, water management, forestry, hydrology, geology and coastal oceanography.

The launch vehicle projects comprise an Augmented Satellite Launch Vehicle (ASLV) and a Polar Satellite Launch Vehicle (PSLV) whose development will rely on the know-how and the subsystems already developed for the Indian launch vehicle SLV-3, which during its first developmental flight in May 1981 put a 38-kilogramme satellite in near-Earth orbit. It is hoped that ASLV will launch a 150-kilogramme satellite in a similar near-Earth orbit by 1983.

The second launch vehicle, PSLV, would be able to launch a 1,000-kilogramme satellite in polar Sun-synchronous orbit by 1987.

The Indian space programme has been quite successful. The first Indian experimental communications satellite, Apple, although one of the solar panels did not function, could be used for about 14 hours a day and will be used for conducting a course for teachers in institutes of technology on the use of satellite networks in university education. This project, like those based on communications satellites still to be launched will draw on the experience of earlier experiments in broadcasting to Indian villages direct.

The first functional telecommunication multi-purpose satellite, INSAT-1A, launched on 10 April 1982, is now ready for use in telecommunications, television broadcasting and weather monitoring although non-deployment of the C-band antenna and the continued malfunctioning of solar sails may curtail its useful life. But this may not matter if INSAT-1B, to be launched in July 1983 by the American space shuttle, functions well. The two satellites, INSAT-1A and INSAT-1B, have a total operational lifetime of 10 years with an overlapping period of two years.

By 1990, the Department of Space may have launched and tested INSAT-2, an improvement on the present INSAT satellite. It may then be necessary to have one satellite for telecommunications and television and one for meteorology.

Zaka Imam

Permanent space station

Europe cautious

The European Space Agency (ESA) is cautiously considering an invitation from the US National Aeronautics and Space Administration (NASA) to collaborate in building a permanent manned space station. NASA believes that international collaboration will improve the chance that Congress will approve the \$3,000–\$5,000 million project. But the agency, wary of collaboration since NASA withdrew most of its promised participation in the joint international solar polar mission, will be seeking strict assurances that NASA will not renege on the new project.

NASA has been for some time considering two proposals for a manned space station. The Johnson Space Center favours an immediate start on a large manned facility, built piecemeal from components transported by the shuttle, that would serve as an operating base in low Earth orbit. The Marshall Space Flight Center has an initially modest plan for a series of small unmanned platforms that could be built up later into a large manned station.

NASA has invited US industry to submit by the end of this month proposals to define the space station more closely. One eight-month study will define user needs and six smaller studies will draw up specific aspects of space station design. NASA plans more detailed studies in 1983 and 1984, and will next month be putting a request for \$16 million to finance them to the Office of Management and Budget for fiscal year 1984.

Meanwhile, NASA has invited other countries, including Canada and Japan as well as those in Europe, to consider what use they might make of a space station. Last week, ESA decided to support a study of European needs and to call on European industry to participate with US counterparts on the six detailed design studies. European industry will be looking for ways in which it can contribute expertise gained through building Spacelab, the modular laboratory to be carried on the shuttle. So far, ESA has set aside \$6 million for these preliminary studies.

It will not be easy for ESA to decide whether to accept NASA's invitation. Acceptance would almost certainly mean abandoning ESA's independent idea for an advanced transportation system on which those member states that value independence are keen. Largely stimulated by French proposals, ESA is now considering a partly reusable launcher that would build on experience gained during development of Ariane. Several companies, including Aerospatiale, Erno and Marconi, are conducting feasibility studies for a launch system that would be capable of carrying manned payloads. Next year, the agency also plans to place contracts for studies on

an "in-orbit infrastructure" that might even rival NASA's plans for a space station, although the scale of the project has yet to be defined. In parallel with ESA's efforts, the Centre National d'Etudes Spatiales (CNES), the French national space agency, is also studying space transportation systems using robots rather than men in space.

ESA plans to assess the results of its own studies and those of CNES in 1984 in time for an operational launch system by the mid-1990s. But NASA's invitation introduces a new dimension. Clearly, the agency must choose between the independent route and collaboration. That will be a difficult political decision that could make the much discussed, although as yet unscheduled, conference of European space ministers particularly opportune.

The NASA invitation will also sharpen the division between those in Europe who advocate almost total independence in space and those who would rather spend their money on hardware for launch systems developed largely with US money. Much will depend on the price of European space independence, certain to be tempered by what assurances the United States can give that the space station project will not be abandoned when funds have been committed. ESA will be looking for an intergovernmental agreement that provides greater security than the memorandum of understanding in force when NASA decided to abandon the spacecraft it was due to launch as part of the international solar polar mission.

Judy Redfearn

US nuclear power

Risk underestimated?

Washington

A Nuclear Regulatory Commission (NRC) study of actual nuclear power plant operations from 1969 to 1979 has concluded that the likelihood of a major accident — one that could lead to severe damage of the reactor core — has been seriously underestimated.

According to the new findings, a major accident could have been expected every 200 to 600 reactor-years during the period under study. The United States has at present 74 commercial reactors, so that translates to one major accident every three to nine years. NRC's 1975 Reactor Safety Study (also known as WASH-1400, or the Rasmussen report), which has been frequently criticized for underestimating the risks of nuclear power, put the frequency of major accidents at one every 20,000 reactor-years. NRC recently set a safety

goal of one every 10,000 reactor-years.

The new study, *Precursors to Potential Severe Core Damage Accidents*, was prepared for NRC by Oak Ridge National Laboratory's Nuclear Operations Center. It sifted through nearly 20,000 "event reports" that plant operators are required to file with the commission, and identified 169 of these as possible "precursors" to a major accident. In only one of these cases — the March 1979 accident at Three Mile Island Reactor 2 — did severe core damage, as defined by the study, actually occur.

In 52 cases, however, the events were considered to hold a significant risk of leading to severe core damage under the right conditions — particularly if emergency back-up systems subsequently failed. The operator reports include reports on all emergency system failures, including those discovered during routine tests; thus it was possible to calculate the frequency of such failures. This information, combined with the frequency of the "precursors", was used to calculate the overall frequency prediction for a major accident.

The director of NRC's Division of Risk Analysis, Robert Bernero, stresses, however, that the uncertainty in this estimate is large. For one thing, the single accident at Three Mile Island is responsible for about half of the frequency estimate. The study also notes that the estimate is on the conservative side; it "could be too low by a factor of two to three or too large by one or two orders of magnitude", according to William Cottrell, director of the Oak Ridge analysis centre.

Nor does the report take into account the equipment modifications and procedural modifications ordered after the Three Mile Island accident. A second report, now in preparation, will analyse 1980–81 event reports, and should provide a clue to how effective these modifications have in fact been.

The discrepancy between this study and the earlier Rasmussen report seems to hinge on two factors. According to Bernero, the most important is that the earlier study had little actual operating data to go on. Its approach was to think up possible accident scenarios and to use known failure rates of components such as pumps and valves. Inevitably, this approach is incomplete. A striking example of the sort of accident that cannot be anticipated was the bizarre sequence of events at Rancho Seco in March 1978 that began with a dropped light bulb and ended with a loss of main feed water to the reactor.

The second factor is that the Rasmussen report seems to have made some mistakes even in the scenarios it did consider.

Bernero says that although there is "general agreement" between the two reports on failure probabilities, the new data show that the Rasmussen report made a "poor fire analysis" and a poor analysis of certain minor loss-of-coolant accidents that result from pump-seal leaks.

It is significant that the new findings did not reveal any pattern of accidents among plants of any particular vendor, architect-engineer, power rating or age. Thirty-eight per cent of the precursor events involved human error.

NRC hurriedly released the study last week after the Critical Mass Energy Project, a Ralph Nader anti-nuclear group, made public a draft of the study.

Stephan Budiansky

Soviet research careers

Pay impediments

Low pay scales are hampering recruitment into Soviet science, according to a Moscow specialist in economics, Dr G. Lakhtin, writing in *Pravda*. The average salary of a scientist, he said, is less than that of a worker in transport or industry. A major overhaul of the pay structure, he says, is necessary if science is to be productive.

The need to implement the results of "scientific and technical progress" in the economy is a frequent theme in the Soviet press. Recently Vadim Trapeznikov, a former deputy chairman of the state committee for science and technology, published in *Pravda* a blistering account of delays and bungling in diffusing the results of research and development to the shop-floor level. Hitherto, however the problem has been treated as one of organization and planning — in particular, of drawing scientists into closer links with industry. Lakhtin, however, pinpoints another basic problem — how should scientists be rewarded?

Not everybody is badly paid. Lakhtin quotes the example of a worker holding the degree of Candidate of Sciences, head of laboratory in a "First Category" institute and with a service record of more than 10 years, who receives 400 rubles a month, "neither more nor less", compared with the national average monthly salary of about 170 rubles.

Soviet salaries are rigidly defined by academic qualification and job. Past attempts to set a salary range for each grade came to nothing, for individual salaries in each range soon drifted back to the mean. As Lakhtin explains, administrators raised the salaries of younger at the expense of the older workers, thus blunting the incentive to improve qualifications and job status.

Academic qualifications play a major part in fixing salaries, and the degree of Doctor of Science can be worth as much as an extra 100 rubles a month. Doctors of Science are, however, relatively rare, owing to what now appears to many Soviet

Accident	Date	The most significant "precursors"
Three Mile Island 2	28 March 1979	Loss of feedwater; open pilot-operated relief valve. Human error involved.
Browns Ferry 1	22 March 1979	Cable tray fire. Human error involved.
Rancho Seco	20 March 1978	Failure of non-nuclear instrumentation; steam generator dryout. Human error involved.

academics as a historical anomaly. During the industrialization of the 1930s, young graduates were recruited into industry without having had time to complete their PhD studies, and the degree of Candidate of Sciences was introduced as a half-way house.

Now, the degree of Doctor of Sciences is rarely awarded before the age of 40, and often only shortly before retirement at 60. Recently there has been some pressure by academics to abolish the Candidate's degree, which would almost certainly result in a cut in pay for Doctors of Science.

Some opponents of the present structure have suggested that pay should be job-related only. This, says Lakhtin, would simply mean that certain jobs would become associated with certain qualifications, so stifling the incentive for self-improvement. Moreover, a Candidate might be an excellent researcher but have little talent for administration. If the doctorate were linked to the post of laboratory head, and such a Candidate went on to take a Doctor's degree, he would have to be promoted to a post for which he had no aptitude.

Work in science, says Lakhtin, is a "complex social phenomenon" and its remuneration is a "knot where economic, social and psychological factors are entwined". Accordingly he refrains from proposing a solution. Lakhtin's article is precisely of the type used to present to the public a proposed change of policy. One of the general principles for discussion floated by Lakhtin must nevertheless have struck an apprehensive chord in the Soviet Union's almost one million strong research force. Science, said Lakhtin, must enjoy priority in pay, even if this means cutting back the total of those employed in science. A scientist who proves unable to pull his weight should not have his pay cut but should be moved to another sphere of activity.

Vera Rich

French university research

Whose strings?

The powerful research and industry minister, M. Jean-Pierre Chevènement, does not control the whole of science in France, it seems. During a recent meeting with French university staff, the ministry of education's director of research, M. Bernard Descomps, let slip that his ministry was considering setting up elected committees that would review university research proposals. Other indications from the ministry suggest that even a national university research council is possible; and none of these bodies would be under M. Chevènement's political control.

A revolution? Not exactly. The committees and the council would assess applications from the universities for research money controlled by the ministry of education, a relatively paltry sum compared with the flood pouring — or

promised — from the ministry of research and industry. But for a typical university, support from the ministry of education can still account for a fifth of the research budget (aside from salaries) and this can be turned to unfashionable subjects out of favour at the research and industry ministry.

The structure of the elected assessment committees, however, and the nature of the elections, have yet to be determined. Ministry staff say the committees should be multidisciplinary and regional, each assessing the science policy of a number of universities; and that they should judge the distribution of ministry cash — and jobs — in areas "orthogonal" to the interest of the big government research institutions such as the Centre National de la Recherche Scientifique. (These institutions have most of their laboratories in universities, but are controlled by Chevènement's ministry.)

So far so good, but it is clear that there will be problems with the committees. For one thing, multidisciplinary committees are likely to be large and unwieldy, and the regional political battles very fierce; and it will not always be easy to separate the politics of the ministry of education from that of the research and industry ministry.

Meanwhile, French biologists have not been slow to exploit another source of research money, also emanating from the ministry of education, and which may or may not be controlled by the elected committees. These are sizeable funds devoted to a particular research theme, changed each year. This year's flavour covers some of the less fashionable sides of biology, from taxonomy to ethology, which the ministry would like to see profit from advances in techniques in the faster-moving biological sciences. To this end, the ministry earlier this year announced grants totalling some FF 2-3 million (the sum is not yet fixed). So far it has received around 400 applications, each representing a group of some 3-10 French biologists.

How will these applications be assessed? As usual, says the ministry of education, in close liaison with CNRS and others of Chevènement's institutions.

In the past this would not have seemed so like sleeping with the lion as it does now. Many of the best French experts are associated with these institutions, and — after all — before President Mitterrand came to power CNRS belonged to the ministry of education itself. At that time, liaison between university policy and CNRS policy was close. Even now, many in the ministry of education would like it to remain so (after all, M. Chevènement has most of the money!). But certain university researchers, worried about the effects of the Chevènement technological wave, might take comfort from a different possibility: that the separation of CNRS from the ministry of education should encourage the establishment of an independent science politics at the ministry, and so work ultimately in the universities' favour.

Robert Walgate

British biotechnology

Public concern

The Porton Laboratory of the Public Health Laboratory Service, once the British government's microbiological defence research establishment, is probably still the most successful publicly supported biotechnology organization in Britain, at least by the criterion of the value of its products sold. Last year, the laboratory sold products worth more than £900,000, and confidently expects to sell more than £1 million worth in the present financial year.

The Porton laboratory seems now to be well through the metamorphosis from sword to ploughshare. Although the British government has traditionally forsworn the use of biological weapons, until five years ago the Porton laboratory was kept occupied on what was described as a



programme of defensive research. By the skin of its teeth, the laboratory survived a period during which closure seemed imminent. Now, people at the laboratory daydream about the possibility that if that crisis had been a little delayed, the laboratory might have become the channel for public investment in biotechnology, now represented principally by the company Celltech, in which the British government has a 40 per cent stake. On the whole, they conclude, they are better off as they are.

Part of the explanation may be that the laboratory is earning something like £2 million towards its total annual cost of £5.2 million, half of that by means of "in-out" contracts with other public organizations. Of the products being sold, the enzyme asparaginase (used with other drugs in the chemotherapy of leukaemia) is the biggest seller, at about £500,000 a year. Earlier attempts to make Porton a major source of restriction enzymes for recombinant-DNA research have, however, been abandoned. The laboratory's strength is in the large-scale production of bacteria and not, it appears, in marketing products in competitive fields.

The laboratory is also the only source in Britain of human growth hormone from

traditional sources — pituitary glands taken from cadavers. After the upheavals in this business brought about by the introduction of payments to mortuary attendants for extracting pituitaries, the Department of Health seems happy with the way things are going. Whether Porton will become a manufacturer of cloned growth hormone, perhaps under licence, remains to be seen. Certainly the laboratory plans to sell its services in helping commercial biotechnology companies scale up procedures.

The staff at Porton seems to have survived the upheavals of the past five years in reasonably good shape. Departures and new arrivals have been reduced to trickles. The scientific staff of 70 is an unusually small proportion of the total of 400 (including janitors), reflecting for example the large numbers of technicians operating semi-production equipment.

Environmental legislation

Problems persist

Washington

As Congress returns this week from its Independence Day recess, it will continue its long drawn out effort to dispose of two controversial scientific issues, the Clean Water Act and the Clean Air Act.

Last month, the Administration introduced its long-awaited proposals for amending the Clean Water Act. Although both industry and environmentalist groups find fault with points in the Administration package (filed as S 2652), the disagreements seem minor. Environmentalist groups object to a proposed relaxation in the current mandatory requirements that industries pretreat any wastes discharged into municipal sewage systems; industry seems more upset by the omissions of the proposed amendments — specifically, that no waivers would be granted to the act's requirements on wetlands protection or on the installation of "best available technology" for the control of hazardous substances in waste streams.

But despite the relative measure of agreement, it has become increasingly clear that Congress will choose to get involved in modifying the act this year. The Senate's environmental pollution subcommittee of the Committee on Environment and Public Works has scheduled hearings for the last two weeks of July. But Congress's heavy schedule of unfinished business, and the reluctance to deal with even mild controversy in an election year may be formidable obstacles to any further steps. An easy way out is available through another bill (S 2590) which would merely authorize continued funding for the act (due to run out on 30 September) while making no changes at all in its substance.

It is even less likely that action will be taken on the rewriting of the Clean Air Act, which has already been bogged down for over a year. Representative John Dingell

(Democrat, Michigan), chairman of the House Energy and Commerce Committee, had earlier this year put together a coalition — with Administration backing — behind a bill (HR 5252) that would significantly ease the air pollution limits imposed on new automobiles. Specifically, it would double carbon monoxide and nitrogen oxide emission limits and make it harder for the Environmental Protection Agency (EPA) to order recalls on new cars not meeting the standards. The bill would also weaken the complex "prevention of significant deterioration" provisions of the act, which are designed to limit pollution increases in already clean regions. The Dingell bill would eliminate these provisions for all areas except National Parks, and would, according to EPA estimates, roughly double the allowable emissions even in these remaining areas.

When the bill reached the full committee, after clearing the health and environment subcommittee, the coalition started getting shaky. In April, Dingell suspended work on the bill.

Meanwhile in the Senate, prospects that a bill will be passed are becoming very dim. Many people, however, consider that may be the best result. Although the "authorization for appropriation" ran out for the act last September, it remains in force, and Congress last year had no trouble appropriating operating funds for EPA that enabled it to continue to enforce the act. And as one committee staff member puts it, everybody gets something from the continued stalemate. Environmentalists get the continued force of the tough old law, undiluted by concessions to industry, while industry — particularly the utility industry — is spared the burden of regulations aimed at curbing acid rain, which is something the Senate committee wants added to the act. There may be growing sentiment for a bill that would do little more than extend the deadlines that the present act sets for states to meet air quality goals.

Stephen Budiansky

Polish martial law

Hope deferred

Martial law in Poland has fulfilled its purpose, police chief General Jozef Bejm observed on Warsaw television last weekend. This has raised hopes that the anniversary of the foundation of the Polish People's Republic on 22 July could see further relaxations of the regulations, and possibly the conversion of the present "state of war" into a civilian state of emergency. Some commentators have suggested that this could lead to at least a partial lifting of US sanctions against the Soviet bloc. Recent events, however, suggest that in Polish academic life any lightening of the restrictions would be little more than cosmetic.

During the past few weeks, in spite of the release of a number of detainees, there have

been disturbing unofficial reports of new internments. Participants at the recent European Physical Society meeting, for example, heard of the internment in May of a young researcher, Mirosław Hamera, from the Physics Institute of the Polish Academy of Sciences in Wrocław, apparently in connection with the demonstrations over the May Day weekend.

Such internments without formal charge are not generally reported in Poland. When formal charges are preferred, however, the publicity is considerable, as in the case of Dr Ryszard Herczynski who last month received a two-year prison sentence for attempting to send to the West materials containing allegedly false information on the current situation and urging Polish scholars to resist martial law.

One of the documents mentioned in the Herczynski case is a "code of conduct" for academics during martial law. The text of this has not reached the West, but appears to urge non-cooperation with the authorities. Meanwhile, a debate has blown up between the new government daily *Rzeczpospolita* and the once-liberal weekly *Polityka* on the growing trend in intellectual life towards "internal emigration" — defined as retreat into purely academic interests.

Apparently prompted by a meeting towards the end of June between General Jaruzelski and Professor Aleksander Gieysztor, chairman of the Polish Academy of Sciences, the academy's Consultative Committee for Social and Scientific Initiatives announced its intention of becoming a platform for discussion between scientists and the authorities. The committee has called on all members and employees of the academy to cooperate with the committee regardless of their past or present affiliations. So far, the response has not been enthusiastic, and last week General Mieczysław Moczar, head of the government's Supreme Auditing Chamber, complained to the Sejm (Parliament) about the "meagre" role being played by Polish science in modernizing the economy.

Similarly there has been a hiatus in the political "verification" of university personnel, which should have been completed by the end of the academic year. Verification procedures have, however, been blocked in universities with resistance ranging from the "mislaying" of the necessary forms to written protests from departmental councils to the Ministry of Science, Higher Education and Technology. Four University of Warsaw department heads categorically refused to "verify" their subordinates.

The timing is important. If the procedures had been completed by the end of term, unverified academics could have been sacked during the vacation without their students' knowledge — and before the entry into force on 1 September of the new Higher Education Act, which assures university autonomy.

Vera Rich

European collaboration

How, where?

European collaboration in science is a little like the bringing up of children: everybody agrees that it should be done well, but few agree on how. This seems to be the general impression left on those attending the symposium on European science organized last Thursday (8 July) in Bonn by the Max-Planck-Gesellschaft and the European Science Foundation. The symposium was intended as a mark of respect for Dr Friedrich Schneider, for much of his life the general secretary of the Max-Planck-Society, who then served as secretary general of the science foundation until 1980. Schneider died last year.

Lord Flowers, rector of Imperial College London and president of the European Science Foundation for six years until 1980, seems to have left the symposium with the most vivid impression with a forceful argument that universities are not ivory towers but, rather, are embedded in society. So, he said, they should be prepared to shoulder their part of the burden of industrial research — and should also welcome present privations as a means of putting right the excesses of the 1960s.

On mechanisms for supporting research, Flowers argued for a clearer division of responsibility between scientists, governments and politicians (parliamentarians). Scientists, his argument went, should give advice on how funds should be distributed within some chosen field, but governments inevitably have a decisive influence in determining which fields should be cultivated. Because in democratic states only "parliamentarians" can hold the ring, "we must educate our politicians". He commended the British Parliamentary and Scientific Committee as one way of doing this.

Professor Herbert Curien, chairman of the Centre National des Etudes Spatiales in Paris and also president of the European Science Foundation, pointed to the benefits but also the disadvantages of international collaboration built on large projects. The European synchrotron radiation project, he said, would be a valuable project for collaboration, but the danger (exemplified by European research centres such as the Community's laboratory at Ispra in Italy) is that "when you have a facility, you need to find a programme".

Professor Reimer Lüst, president of the Max-Planck-Gesellschaft and chairman of the Schneider symposium, argued, however, that every opportunity for collaboration should be seized, citing as an example the successful collaboration between his organization and the Centre National de la Recherche Scientifique in the high-field magnetic laboratory at Grenoble, on which each organization could have embarked successfully on its own but where the collaborative laboratory was better than either would have been.

Schneider's own work until his death was devoted largely to persuading European governments to collaborate on the synchrotron radiation project, with little success. Schneider, a man with an exceedingly dry wit who was also a famous gourmet, would not have been surprised that Vicomte Etienne Davignon, the commissioner for research at the European Commission, did not arrive to deliver his promised contribution at the symposium. "He's always doing this", said one of the organizers.

Foot and mouth disease

Race to vaccine

The race to produce a synthetic foot and mouth vaccine to replace the use of inactivated virus is still wide open. The United States company Genentech, like other biotechnology companies, is putting its money on genetically engineered virus polypeptide VP1. The Wellcome Foundation in Britain, one of the principal manufacturers of inactivated virus vaccine, is, however, backing both VP1 and the synthetic peptide portion of it now known to be immunologically effective (see *Nature* 1 July, p.30).

The United States Department of Agriculture last year raised hopes that genetically engineered VP1, the main antigenic determinant of the virus, would be the answer. But the department's announcement has been criticized as premature. For while it seems that genetically engineered *E.coli* can be a prolific source of VP1, the intact polypeptide is not as immunologically effective as expected. The synthetic peptides, corresponding to amino acids 141–160 within the VP1 polypeptide, seemed more promising when described earlier this month.

Genentech, working under an agreement with the Plum Island Laboratory of the US Department of Agriculture, is not convinced that peptides are the only feasible route to a synthetic vaccine. The company now claims that it has achieved similar protection with VP1 polypeptide in small mammals by an improved method of extraction and purification. Dr Dennis Kleid, head of the project at Genentech, says that the new technique does not denature the VP1 polypeptide.

Genentech and Plum Island have apparently refined their recombinant DNA technique to produce 2×10^6 VP1 molecules per bacterium. Genentech says that it is growing genetically engineered *E.coli* in a 10-litre fermenter and purifying enough VP1 for trials in cattle at Plum Island. Dr Kleid hopes that scale-up will be quick, and that field trials can be mounted in South America, one of the largest markets for foot and mouth vaccine, late this year or early next. A commercial vaccine, says Kleid, could be ready some time in the mid-1980s. Genentech is

working on the problems of scale-up with the International Minerals and Chemical Corporation of Indiana, which has already been licensed for commercial production.

The Wellcome Foundation, however, does not share Genentech's optimism about VP1 polypeptide. Although the foundation will keep an interest in VP1, it plans to spread its risks by exploring the possibilities of a commercial peptide vaccine. But the economics of a synthetic vaccine, of whatever type, may not appear the same to the Wellcome Foundation as to Genentech and the International Minerals and Chemical Corporation.

Wellcome already manufactures foot and mouth vaccine on a large scale by inactivating virus grown in cell culture. Individual doses cost just a few cents. A synthetic vaccine will have to be markedly cheaper to persuade the company to convert its existing manufacturing processes, according to Thomas Pay, scientific coordinator of the foundation's foot and mouth disease technical division. Pay is also sceptical of arguments that synthetic vaccines would be safer than those based on inactivated virus. He says that inactivation has been improved by avoiding the use of formaldehyde.

In the meantime, neither Wellcome nor Genentech believes that the prospect of a synthetic vaccine should hold up foot and mouth eradication programmes. That view is shared by the Food and Agriculture Organization of the United Nations, which says that the chief practical effect of a synthetic vaccine will be to do away with the need to refrigerate vaccine stocks — hardly a requirement to delay eradication programmes.

Judy Redfearn

Developing countries

European split

Brussels

The European Parliament has given the European Commission's research and development programme on behalf of the developing countries a hostile reception. The Parliament last month ridiculed the programme on the grounds that it was too small, for its choice of priorities and because the chief beneficiaries would be research institutions in the Community and not scientists in developing countries.

The Commission's proposal is to spend 40 million units of account (£70 million). The debate took place against the background of heated arguments over the Community's new world hunger strategy and the attempts by the development commissioner, Edgard Pisani, to redirect development aid from large construction projects to grass-roots stimulation of the poorest economies.

The Commission admits that the funds available will not make a substantial contribution in the areas regarded as most important for the developing countries — nutrition, health, energy and natural

resources. It therefore selected narrower objectives, including transmissible tropical diseases, mother and child care, genetics, environmental hygiene and nutrition and tropical agriculture.

The European Parliament last month lambasted the Commission for the lack of consultation with the countries intended to benefit and the failure to coordinate plans with those of the World Health Organization (in tropical medicine) and the Consultative Group for International Agricultural Research.

The Parliament's view is that the programme takes too little account of the need for training in research, and of the need to develop career structures for scientists. The Parliament is especially critical of the way in which the Commission plans to spread its meagre resources thinly over too many projects.

The Parliament's criticisms are embodied in a report by one of its West German members, Renate Rabbethy. To the extent that the report advocates that Community spending on development assistance should not become a hidden subsidy for European institutes, the Parliament has made common cause with Mr Pisani. What will eventually happen to the Community's development research programme will depend also on the reaction of the commissioner for research, Vicomte Etienne Davignon, who is also said to favour a more generous programme.

Jasper Becker

Pharmaceuticals in Bangladesh

Use less drugs

The government of Bangladesh, in a move that has delighted voluntary agencies but upset many of the pharmaceutical companies operating there, has banned 1,742 drugs described as "harmful" or "unnecessary" as part of a wide-ranging new drug policy. On 12 June the Ministry of Health published a new Drug Ordinance, replacing the Drug Act of 1940, which prohibits the sale of 237 "harmful" drugs with immediate effect and of 1,505 other "unnecessary" products which are to be withdrawn or reformulated within six months.

The new ordinance follows the advice of an Expert Committee on Drugs and Drugs Policy which met earlier this year under the chairmanship of Professor Nurul Islam. The committee's report was accepted by a council of advisers in May. The objective of the new drugs policy, according to the committee's report, is "to ensure the quality and availability of essential drugs which are of relevance to the health needs of the majority of the population". The report refers to the need for companies to concentrate on a limited list of 150 essential drugs as recommended by the World Health Organization. Limitations on national resources and the shortage of foreign exchange are given as reasons for

the removal of "all unnecessary, useless drugs and drugs of doubtful efficacy".

Prominent on the banned list are tonics, cough linctuses and antibiotic tetracycline syrups such as Glaxo's Clinmycin syrup and Pfizer's Vibramycin syrup, stated to be harmful to the bony growth of children — although tetracycline in capsule form may still be sold for adult use. Hoechst's Polytamin, a combination vitamin tonic which includes vitamin B₁₂ and alcohol, is described as "one of the most abused drugs on the market"; and Squibb's Verdaviton Elixir is said to be "a highly misused, dangerous, habit-forming drug".

Also listed are antidiarrhoeal drugs already either banned or withdrawn from the market in the United States and European countries, such as Ciba-Geigy's Enterovioform and Mexaform tablets.

The government's initiative has been welcomed by voluntary health organizations in Bangladesh which have for several years drawn attention to the dangers of inappropriate drugs circulating in Bangladesh without doctor's prescriptions and at high cost. One organization, Gonoshasthaya Kendra (the People's Health Centre), which provides health services to a population of 100,000 at Savar, near Dacca, has even been manufacturing its own low-cost "essential" drugs as alternatives to the products of large companies. This venture is supported by Dutch and British voluntary agencies, including Oxfam and Christian Aid, which recently made a further grant of £25,000 towards its operating costs.

The more immediate impact of the new ordinance will be on the profitability of the 166 licensed manufacturing companies. About 75 per cent of the pharmaceutical market in Bangladesh is controlled by eight foreign-based companies, of which four are in Britain: Fisons, Glaxo, ICI and May & Baker. According to the Consumer Association of Bangladesh, these four companies had a turnover in 1981 of 420 million taka (about £10 million). Twenty-five local companies supplied a further 15 per cent of the market.

The new drug policy will not easily be implemented. The smaller producers and exporters, as well as the medium-sized companies, are seriously threatened. At an emergency meeting in Dacca in June of the Bangladesh Aushad Shilpa Samity, an association of 33 pharmaceutical companies including six multinationals, it was claimed that local production of medicines would drop by 80 per cent if the new policy were implemented and that thousands of jobs would be sacrificed.

In a carefully-worded appeal to the Martial Law Authority published in Bangladesh newspapers, the Samity gave its "full support" to the review of drugs and medicines as part of the health-care system but went on to say that national rather than multinational companies could be hardest hit.

John Montagu

Diderot's mantle for Chevenement?

A "tremendous project" to launch an "encyclopaedia for the 21st century" is under way at the French ministry of research and industry. It springs from the enthusiasm of the minister, Jean-Pierre Chevènement, for bolstering French as a language of science.

So far, no details have been fixed, but other ministers have warmly approved a confidential report on the subject. In the National Assembly recently, Chevènement described the project as "a great cultural enterprise inspired by the encyclopaedia of Diderot and d'Alembert". He reckons that the new encyclopaedia could be completed by 1989 — too late for the next presidential election.

Diderot and d'Alembert's encyclopaedia played an important role in the "Enlightenment", the period of unbounded faith in science and reason that Chevènement would dearly like to see reborn in the new France. More-

"And a free French dictionary with every set!"



over, today an encyclopaedia need not appear only in print: "books, pamphlets, pictures, films, videotapes, videodisks, all the means of modern technology would be used to place at the disposition of France and all those who speak French... the knowledge of humanity", the minister told the French deputies.

Although the encyclopaedia would be likely to concentrate on science, technology and the arts of manufacture, it would have a very open structure — where anyone who wanted to contribute could do so (subject to a degree of vetting by a committee of specialists). The objective, according to a spokesman for the ministerial information agency, MIDIST, which will co-ordinate the project, is to create a "living" encyclopaedia that would reflect many shades of opinion.

Questions of cost, manpower and other such practical matters have yet, however, to be answered. "All we have is the proposal and a political will to go forward" said the MIDIST spokesman.

Robert Walgate

CORRESPONDENCE

Access to journals

SIR — Your recent offer allowing scientists outside Poland to purchase, on behalf of a Polish colleague, a subscription to *Nature* at a reduced rate (*Nature* in Poland, 3 June p.354) is of commendable generosity. Respecting your desire to help Polish science we should like, nevertheless, to point out that the reported current deprivations suffered by Polish researchers are but a permanent and frustrating way of life for thousands of scientists throughout the developing world, for whom access to *Nature* and other scientific journals is invariably uncertain. The fact that their difficulties lack the publicized polemic attendant upon the current situation in Poland should in no way diminish our interest in their considerable scientific needs.

Nature clearly has a role to play as a detached observer of the repercussions felt in scientific circles consequent upon more general political events. We feel, however, that the selective character of your gesture, although consonant with the unabashed, and at times highly coloured, editorial commentaries in your columns, might compromise the impartiality to which you presumably aspire.

We urge you therefore to extend your offer to all those countries of the developing world where scarcity of hard currency hinders the purchase of individual, and indeed general, subscriptions to *Nature*, thereby affirming your commitment to the dispassion so precious in a serious scientific journal.

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THE circumstances that have recently arisen in Poland are special and, it is hoped, may be temporary and justify the special arrangements made — Editor, *Nature*.

Alternative to war

SIR — The pages of *Nature* continue to describe the arms race, yet no one writes of positive alternatives. The people, American or internationally, feel futile and sense issues are beyond their own influence. Point and counter-point by their leadership go on without the average family understanding the increments of escalation, yet reading that 100 million of "them" could die on the first day of nuclear bomb exchange.

The dilemma which feeds the arms race is that no dependable defence against nuclear weapons except more nuclear weapons has been conceived. Perhaps though, the annals of history may have modern application.

For thousands of years, kings, sultans, emperors, khans, used marriage as a form of national security; opposing tribes, federations, nations, reasoned that no one has more precious treasure than a daughter, and placing daughters by formal marriage, one or more brides in the capital of the opposition, was equally a means of communication and a surety bond. Diplomacy through romance was an effective, inexpensive form of defence.

Why not a modern application of this idea? Why not neutralize nuclear attack not by stockpiling "over-kill", but by an exchange of the two nations' best young women and men, not through arranged marriages, although that may well be a "fall-out" from this kind of defence weapon. Why not negotiate an International Peace Army, equal numbers of Russians and Americans, equal numbers of women and men, all with or getting a college education, all single, all selected by national lottery, all between 20 and 25, all required to live one year in the other's country? Why not 250,000, in distinctive, attractive uniforms, in each country's universities from September to June each academic year, and then a three-month tour of each other's country?

Why not require that among these exchanged scholars be a defined percentage of the children or grandchildren of each nation's generals, congressmen, senators, central committee, political bureau, and cabinet?

This plan offers all merits and no liabilities. Both countries have good university systems, and an accredited year of university education and experience in a major foreign country, with full financial support, with such peace army service in lieu of traditional military draft, would not be an unreasonable sacrifice for a nation's youth. The assurance that another man will not harm his children and grandchildren is almost as basic a human value as can be defined. Such treasure placed in each country would give nuclear weapon reduction a new logic.

Among the assurances needed from members of an international peace army is that all must return to their home country. Some latitude for the success of propinquity, with consequent love, marriage and eventually children, will need to be anticipated. The most dedicated nuclear-response senator or central committee member may be softened by the presence of a son, daughter-in-law, and grandchildren living at ground-zero, abroad.

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Nuclear risks

SIR — I really cannot leave the statements made by Robert Yaes (*Nature* 24 June, p.622) unchallenged. He comments that "It is naive to compare this [the many thousands of people killed by dam failures] to the record of the nuclear power industry". It might be "naive" if the comparisons were unsupported by a thorough analysis of nuclear risks, but that is not so. The searching nuclear risk assessments of the past decade justify regarding the nuclear safety record, which is second to none, as the proof of the pudding, not just a fluke. Even Dr Yaes's so-called "near misses", including Three Mile Island, serve to demonstrate the adequacy of the meticulous precautions taken in reactor design, and to vindicate the essential soundness of the defence in depth strategy adopted.

For many current industrial and commercial activities, including generating electricity from nuclear power, it is just conceivable, so long as one assumes a total failure of highly reliable safety devices coinciding with extremely improbable external conditions, that an accident could cause a large number of deaths.

However, I know of no single nuclear reactor accident thought remotely capable of killing "several million people" as Dr Yaes so irresponsibly suggests.

As Sir Walter Marshall indicated in his recent lecture to the Royal Society (29 June), headlines such as "Reactor incident could kill 10,000 people in London" are completely misleading. "Could" in this instance means that such an accident is imaginable as a very remote possibility. It does not mean that such accidents are a "real" possibility (in the sense that two jumbo jets colliding is a "real" possibility). Furthermore the word "kill" is not to be interpreted as the sudden and immediate deaths resulting from dam disasters or earthquakes. Rather it must be viewed as an "adverse health effect" on a par with that due to cigarette smoking. Given that it did in fact occur, it can be equated to requiring the exposed population to smoke 1/20th of a cigarette each week over the period 10 to 40 years after the accident — hardly the picture Dr Yaes's letter conveys.

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Be fair to parasites

SIR — In a recent letter (*Nature* 4 March, p.10), Professor F.E.G. Cox states that "infections with parasites are characteristically long and chronic and the resulting diseases involve diverse pathological mechanisms. The damage caused is vastly in excess of the expected local reactions to the parasites themselves and appears out of all proportion to their size." I find this generalization to be both lacking in breadth of meaning and inaccurate in content.

The original meaning of parasite stems from the Greek *parasitos*, which means one who eats at another's table. Like a capricious bandit who has forced his way in, the parasite now obligates the host to prepare a banquet for him. This statement is made without reference to pathogenicity and diseases caused by parasites are usually a function of parasite density and the pathological state or health of the host. From the standpoint of its survival and species perpetuation, it would be deleterious to the parasite to cause the death of the host. The adverse cellular reactions which can accompany parasitism usually suggest a comparatively recent relationship in evolutionary time.

Some parasites may even be of benefit. The host may gain from the exchange of chemical substances with the parasite, or a species of nonpathogenic parasite may protect the host from infection by another, perhaps more injurious, species. It is estimated that *Entamoeba histolytica* is found in faeces of over 400 million people throughout the world, but most of the time these organisms lead a nonpathogenic existence in the intestine. Only when certain physiologic changes take place in the host does *E. histolytica* become pathogenic.

Relationships between parasites and hosts are complex, and their associations are as varied and unique as the individual components that make up each system.

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NEWS AND VIEWS

Opioid peptides have found their roots

from Jean Rossier

JUST a few months after the full characterization of the enkephalin precursor¹⁻³, Numa's group at the University of Kyoto strikes again with the publication, in this issue of *Nature* (p.245), of the sequence of the common precursor for dynorphin and the neo-endorphins⁴. In 1979 this same group was the first to come up with the complete sequence of proopiometanocortin⁵, the common precursor for β -endorphin (another opioid peptide), ACTH (corticotrophin, the hormone responsible for the secretion of corticoids by the adrenals) and the MSHs (melanotropins). All of these sequencing data were obtained by cDNA technologies that are now showing such promise for the neurosciences.

Numa's group has thus obtained the sequence of three different precursors which contain all of the different opioid peptides characterized so far. Since 1975, when Hughes *et al.*⁶ demonstrated that the pig brain contained two peptides, Met-enkephalin and Leu-enkephalin, which appeared to be endogenous ligands for opiate receptors, the list of such compounds has grown tremendously (see the figure).

At the time of the discovery of Met-enkephalin, it was observed that its sequence was also present in a large pituitary peptide, β -lipotropin. Concurrently, several groups found that the β -lipotropin C-terminal fragment starting with the sequence of Met-enkephalin had very potent opiate activity. This 31 residue C-fragment was renamed β -endorphin.

A few years later, Avram Goldstein (University of Stanford) discovered that the pituitary contained another very potent opioid peptide, which was named dynorphin⁷. The complete sequence of this 17 amino acid peptide was elucidated last year^{7,8}. At around the same time, two other opioid peptides with properties similar to dynorphin were discovered by Matsuo's group (Miyasaki, Japan)^{9,10}. These peptides, α - and β -neo-endorphin, were isolated from swine hypothalami and share with dynorphin a common N-terminal Leu-enkephalin sequence. β -neo-

endorphin has the structure of α -neo-endorphin with the deletion of the last C-terminal amino acid.

For a while it was generally accepted that dynorphin and the neo-endorphins were precursors for Leu-enkephalin. It was also thought that β -endorphin was the precursor for Met-enkephalin. These hypotheses had, however, to be rejected when it was found that the anatomical distribution of the putative precursors did not parallel that of the enkephalins¹¹.

Recent work with cDNA technologies has now clarified the precursor relationship of these opioid peptides. Indeed, three completely different precursors with molecular weights around 28,000 have now been characterized and sequenced. The family tree of endogenous opiates seems to possess three main branches. The branch containing β -endorphin and its fragments (α -, γ - and des-Tyr- γ -endorphin) stems from proopiometanocortin. This molecule is found predominantly in the pituitary but has also been found in the brain.

The second branch is that of the enkephalins, whose precursor is the adrenal proenkephalin. This branch no longer just contains Met- and Leu-enkephalins. cDNA sequencing has shown that the adrenal precursor contains four copies of Met-enkephalin, one copy each of Leu-enkephalin, Met-enkephalin-Arg⁶-Phe⁷ (heptapeptide) and Met-enkephalin-Arg⁶-Gly⁷-Leu⁸ (octapeptide¹⁻³). Since the

discovery of the hepta- and octapeptides in the adrenal, it has now been shown by Patey (CNRS, Paris) that these peptides are present in and released from rat brain slices by depolarization. Such results, and the recent identification by Herbert's group (University of Oregon) of brain mRNA that hybridizes with the adrenal proenkephalin, indicate that the brain proenkephalin is similar to the adrenal protein.

The third and most recently discovered branch is that of dynorphin and the neo-endorphins. The paper of Numa's group in this issue of *Nature* may reveal that this branch is not limited to neo-endorphin and dynorphin but may also include a new dynorphin-like molecule. Indeed, the precursor characterized by Numa contains three copies of Leu-enkephalin. Two of these correspond to neo-endorphin and dynorphin. The third may correspond to a new dynorphin molecule that would be formed by the 29 C-terminal amino acids of the precursor. This new dynorphin would also start with a Leu-enkephalin sequence and it may simply give rise to Leu-enkephalin.

The demonstration using cDNA technology that neo-endorphin and dynorphin are synthesized from the same precursor gives biochemical support to the recent cytological observation by Weber (Stanford University School of Medicine) that neo-endorphin immunoreactivity and dynorphin immunoreactivity are localized

AMINO ACID SEQUENCES OF THE OPIOID PEPTIDES		
PRECURSORS	PEPTIDES	STRUCTURES
Pro-opio-melanocortin	alpha-endorphin	Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr ₁₆
	gamma-endorphin	Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu ₁₂
	beta-endorphin	Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-His-Lys-Lys-Gly-Gln ₃₁
Proenkephalin	Met-enkephalin	Tyr-Gly-Gly-Phe-Met ₅
	Leu-enkephalin	Tyr-Gly-Gly-Phe-Leu ₅
	Octapeptide	Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu ₈
	Heptapeptide	Tyr-Gly-Gly-Phe-Met-Arg-Phe ₇
Prodynorphin *	Dynorphin (1-17)	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln ₁₇
	Dynorphin (1-8)	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile ₈
	alpha-neo-endorphin	Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro-Lys ₁₀
	beta-neo-endorphin	Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro ₉
	New dynorphin ?	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Gln-Phe-Lys-Val-Val-Thr-Arg-Ser-Gln-Glu-Asp-Pro-Asn-Ala-Tyr-Tyr-Glu-Glu-Leu-Phe-Asp-Val ₂₉

On the left of the sequence Tyr is always the first amino acid. It has a free amino group not involved in a peptide bond and forms the amino terminal (N-terminus). The other end of the sequence with a free carboxyl group is the carboxy terminal (C-terminus). *Numa (see p.245) uses the term proenkephalin B for this precursor. As explained in the text, I suggest 'prodynorphin' may be more appropriate.

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in the same cells of the hypothalamus¹². Moreover, Weber also has some circumstantial evidence that dynorphin immunoreactivity and α -neo-endorphin immunoreactivity are present in equimolar amounts in various parts of the brain¹³.

In his report, Numa has used the term proenkephalin B for the neo-endorphin/dynorphin precursor. To avoid confusion with the previously characterized precursor for enkephalin (Met-, Leu-enkephalin, octapeptide and heptapeptide), I would propose another name for this molecule: prodynorphin. To follow current practices, prodynorphin would designate prodynorphin with the signal peptide.

Since the discovery of dynorphin and neo-endorphin, pharmacologists have searched for their specific role. At present it is known that dynorphin and the other C-terminally elongated Leu-enkephalins bind quite specifically to one subclass of opiate receptors, the κ -receptor¹⁴. The two other subclasses are the μ -receptor, whose endogenous ligands appear to be β -endorphin and the enkephalins, and the δ -receptor, with enkephalins for ligands. Nevertheless, the characterization of these binding sites has not yet clarified the relationships between these different receptors and the various physiological roles of the different opioid peptides. This question is not at all clear for any of the receptor subclasses except, perhaps, for the μ -receptor, which seems to be strongly correlated with central analgesia.

The similarities between the three opioid peptide precursors are striking. They all contain multiple neurohormonal units in the carboxy-terminal region and the amino-terminal region is a cysteine-rich sequence preceded by a signal peptide. In proenkephalin and prodynorphin, the amino-terminal regions show considerable homology, with potential disulphide bridges located at identical intervals. This marked homology may form the basis for a generalized model for neuronal prohormones and suggests, in addition, that proper folding of this region may be important for correct processing of the prohormones into their final products.

The characterization of the enzymes involved in this process is still in limbo. Such enzymes may have unique specificities. Indeed, all the peptides derived from prodynorphin contain a pair of basic amino acid residues and current dogma dictates that such a sequence provides a signal for trypsin-like proteases. Following the characterization of prodynorphin, we should now consider other processing

strategies.

At present, we do not know in which form dynorphin is released. It may be dynorphin₁₋₁₇, dynorphin₁₋₈, or smaller peptides. The same question exists for α -neo-endorphin, β -neo-endorphin and the new dynorphin present at the C-terminus of prodynorphin. The answer to these questions may come from the elucidation of the processing mechanisms.

Is the marrow stroma transplantable?

from T.M. Dexter

It has long been assumed that the success of a marrow transplant depends largely on the ability of the transfused blood stem cells to proliferate and differentiate among the stromal cells which make up the framework of the host haematopoietic tissue. Relatively little attention has been paid to the possibility that the stromal cells themselves may be transplantable, or to the possible significance of this for long-term survival following marrow transplantation. The work now reported by Keating *et al.* (this issue of *Nature*, p.281) indicates that marrow stromal cell precursors are indeed transplantable, that these include cells with endothelial characteristics and that such cells increase in number with time after transplantation. The result may be of considerable importance for it implies that, in some cases, successful transplantation may depend not only on the transfusion of blood stem cells but also on the transfusion and engraftment of stromal cell precursors.

Haematopoiesis *in vivo* occurs in association with a variety of cells collectively termed the marrow stroma. Several stromal cell types can be easily recognized: endothelial cells which line the marrow sinuses, adventitial reticular cells which branch out into the marrow spaces and enclose areas of haematopoiesis, marrow adipocytes and macrophages¹. Studies *in vitro* indicate the importance of intimate contact between stromal and haematopoietic cells and observations both *in vivo* and *in vitro* show consistent associations between the various haematopoietic cell lineages and discrete stromal elements. For example, granulopoiesis *in vivo* occurs in association with alkaline-positive extravascular reticular cells and erythropoiesis with the sinusoidal endothelium and macrophages².

In long-term marrow cultures, haematopoietic stem cell proliferation and differentiation can only be maintained if marrow-derived adherent cells are present³. Studies using long-term marrow cultures *in vitro* emphasize the importance of this marrow 'stroma' in maintaining haematopoiesis

and have shown similar associations between alkaline phosphatase preadipocyte cells and granulocytes and between endothelial-like cells, macrophages and developing red cells⁴. In fact, in all aspects so far studied, the haematopoietic cells produced in long-term culture are indistinguishable from their counterparts produced *in vivo* and because such cultures mimic haematopoiesis *in vivo* they have been taken as a suitable model for studying marrow stroma structure and function.

In the work reported by Keating *et al.* in this issue of *Nature*, long-term cultures have been established from human marrow transplant patients with the object of determining whether the cells which provide the inductive environment *in vitro* are derived from the patient or the transplanted donor cells. Fourteen patients received a marrow transplant from opposite-sex siblings and various times after transplantation marrow aspirates were established in long-term cultures. The origin of the stromal cells was determined by fluorescent Y-body analysis which makes it possible to detect the (male) Y-chromosome. The percentage of donor cells contributing to the culture stromal environment progressively increased in marrow aspirates taken at greater times after transplantation. In some cases where cells were taken from the patient 40 days after transplantation, practically all the cells were of donor origin.

These data apparently conflict with other studies showing that cultured, cloned fibroblast cell lines derived from transplanted patients are of host origin⁵. However, the role of the fibroblasts is not clear since they are unable to support *in vitro* haematopoiesis and do not seem to be a significant component in actively haematopoietic long-term cultures⁶. Nonetheless, in a recent study⁷, long-term culture adherent cell layers derived from irradiated mice transplanted with

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chromosomally marked marrow cells (and containing the diverse cell phenotypes associated with a competent inductive environment) were shown to be of host rather than donor origin. Do mice and men really differ or is there some technical explanation for this contradiction?

A major difference is in the conditioning of the recipients before transplantation. In Keating's study, the patients were pre-treated with cyclophosphamide and radiation while only radiation ablation was used with the mice. Haematopoietic stroma *in situ* is known to be relatively radio-resistant but its response to cyclophosphamide has not been clearly charted. Is it possible, then, that the cyclophosphamide is 'punching holes' in the marrow stroma and thus allowing migrating stromal cell precursors of donor origin to lodge and proliferate? Then there is the cell dose used for transplantation — with only minimal numbers of haematopoietic cells being transplanted in the murine study it is possible that the number of stromal cell precursors transplanted was insufficient to establish detectable stroma chimaerism. And, of course, there is the time interval. The Seattle study clearly shows a progressive increase in donor stromal elements with time after transplantation — similar studies have not yet been performed in mice.

A major argument, however, will no doubt take place about the nature and the origin of the adherent cells developing in the human cultures. Three questions immediately arise. First, are the adherent cells really 'stroma' or are they a population of adherent haematopoietic cells (expected to be of donor origin)? In this regard, analysis has shown that up to 25 per cent of the adherent cells in some cultures were positive for factor VIII-associated antigen and also synthesized basal (type IV) collagen as well as interstitial (type III) collagen. These data indicate that at least part of the adherent cell population has an endothelial-like nature. Significantly, non-specific esterase-positive cells were not an important component of the adherent cell population, indicating that macrophages comprised only a minority population. This is particularly important, since macrophages are known to be derived from the haematopoietic stem cell via a committed granulocyte macrophage progenitor cell and of course they would be expected to be

of donor origin⁸. Second, the Keating study has shown only that stromal precursor cells are present in the transplanted recipient, that is, cells with the capacity to proliferate *in vitro* and form foci of adherent cells. There is no evidence that a similar proliferation occurs *in vivo* and that the host marrow indeed becomes infiltrated with mature stromal elements. *In situ* analysis is required. Third, do the stromal cells and the haematopoietic cells share a common stem cell as suggested? This is perhaps the most controversial point of all and would imply that a common stem cell can produce both the pluripotential haematopoietic cells and progenitor cells, as well as the stromal cells which are necessary for their maintenance and regulation — a complicated system indeed. However, work in support of this hypothesis was presented by Fialkow at a recent meeting in Copenhagen⁹. Fialkow, Singer and their collaborators have used the glucose-6-phosphate dehydrogenase (G6PD) X-chromosome-linked isoenzyme system¹⁰ to show that in cultures of a chronic myelocytic leukaemia (CML) patient, heterozygous for G6PD types A and B, the CML haematopoietic cells and

the adherent stroma produced in long-term cultures were of the same isoenzyme type (indicating that they may both be derived from the same 'transformed' clone) whereas the marrow fibroblast had a separate stromal origin possessing both isoenzyme types.

What is the significance of the Keating study for marrow transplantation? The intimate cell association seen in long-term cultures and in haematopoietic islands *in vivo* indicates the importance of cell contact in haematopoietic repopulation. Such contact may involve self-recognition signals of the types described for interactions between lymphocytes and macrophages, that is, some degree of H-2 compatibility in mice or HLA matching in humans is required for stromal cell-haematopoietic cell interactions. In this context, there is confusion in the literature regarding the success or otherwise of allogeneic non-matched marrow transplants. In these cases, is it possible that successful transplantation requires transfusion not only of haematopoietic stem cells but also transfusion and engraftment of stromal cell precursors? We will no doubt know the answer in the near future. □

100 to 200 year solar periodicities

from Austin Long

EVIDENCE is mounting for the existence of medium-term solar periodicities. The Sun is the primary source of energy on the Earth's surface, and the fact that different parts of the Earth receive more or less of this energy determines the differences in present-day climate. No one seriously questions these statements, but the causal factors of the major temporal climatic changes the Earth has experienced, and especially the more subtle regional changes in climate, remain controversial. The most compelling evidence is for the triggering of the major glacial-interglacial oscillations characterizing the Pleistocene epoch by the regular and calculable periodicities in orbital parameters.

Less dramatic regional climate variations have also occurred on shorter time scales. For example, many parts of the Earth seem to have experienced a mid-Holocene warming some 4,000–8,000 years ago. In Northern Europe and North America, historians record a relatively cool period during the 16th and 17th centuries. Again it is tempting to look to the Sun for a cause. Basic to such a case is the question of variability in the Sun's energy output.

Satellite-based measurements have recently confirmed what sunspot observers have long suspected: the Sun's energy output does vary. Evidence for variations associated with the 27 day rotational period is convincing; that for the existence of

output variations associated with the sunspot variations (recent results of Wilson *et al.*²) is more controversial (see refs 3 & 4). Examination of longer-term variations requires proxy techniques, of which the most promising so far is measurement of the variability of ¹⁴C production rate. Cosmic ray-induced neutrons produce ¹⁴C by reaction with ¹⁴N, both in the stratosphere and troposphere. Fluctuations in the solar wind intensity modulate the geomagnetic field and hence its cosmic-ray shielding efficiency³. Solar energy output thus affects ¹⁴C production on Earth. Long ¹⁴C production rate records require an accurately dated, precisely measurable sample of past atmospheric CO₂. In certain special circumstances, tree rings can fulfill all these requirements.

Using wood dated dendrochronologically and supplied by C.W. Ferguson of Arizona's Laboratory of Tree-Ring Research⁶, several groups have measured ¹⁴C activity on decadal samples (summarized in ref. 7). Neftel *et al.*⁸ have applied two different smoothing techniques to the 8,000 year span of data from the La Jolla laboratory and obtained power spectra. One hundred and fifty to two hundred year periodicities emerged above the 99 per cent

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confidence curve. Solar wind variations seem likely to have caused these periodic ^{14}C production fluctuations. DeJong and Mook⁹, using high-precision ^{14}C counting equipment, observed a 150 year periodicity over a 700 year time span in the 4th millennium BC. Stuiver¹⁰ finds similar oscillations in wood grown in the last two thousand years with periodicities of about 130 and 200 years. The similar shapes of six ^{14}C production rate curves suggests that such long-term regularities in the ^{14}C production limit the randomness implied by some solar dynamo theories. Stuiver further points out that these periodicities, about 130 and 200 years, show up in several climate parameters. The postulated relationships between solar activity and climate on short, medium and long time scales have been well summarized by Wigley² but the correlation between solar activity and climate is controversial¹¹⁻¹³.

Periodic, non-random solar climate behaviour may not be a geologically recent phenomenon¹⁴ as strong 11, 22, 145 and 290 year periodicities and a weaker 90 year periodicity have been found in the thickness and colour of clastic laminated sediments from Precambrian glacial varves in Australia about 680 million years old. If these deposits are indeed annual (again controversial, see ref. 15), then the apparent long-term persistence of 130-150 year periodic oscillations in ^{14}C production and in climate implies long-term persistence of the instability mechanism which gives rise to the periodicities (see refs 10, 16 for discussion of periodicities in the Sun).

Solar periodicities, inferred from ^{14}C production rates, will very likely extend into the late Pleistocene as tree ring chronologies from the western US and northern Europe continue to lengthen. Accelerator mass spectrometer determinations presented at the Argonne conference¹⁷ of both ^{14}C and ^{10}Be , another cosmogenic radioisotope, in ice cores will further extend the time range of inferred solar activity. These types of data may eventually be the threads that link solar processes and secular climate changes, and eventually lead to a better understanding of both, even though the mechanism still eludes us. □

Unscrambling amblyopia

from Oliver Braddick

HUMANS with normal vision have a remarkable ability to detect a small break where two parts of a line are misaligned. This 'hyperacuity' has now been tested in those suffering from amblyopia — the most common cause of visual loss in childhood — and different acuity losses have been found associated with different amblyopias (see this issue of *Nature*, p.268).

Amblyopia is of particular interest because it appears to be a disorder of the neural processes of vision rather than of the physiological processes that support vision. It cannot be explained by any visible pathology in the eye itself and it cannot be corrected by any spectacle lens. Amblyopia arises when one eye becomes 'disused', either because it focuses less well than the other eye ('anisometropic amblyopia') or because it has been misaligned relative to the other eye ('strabismic amblyopia') or both.

The 'poor vision' of the amblyopic eye has usually been clinically assessed by the acuity shown in reading the familiar Snellen letter chart. Psychophysicists have wanted to explore the nature of the deficit more fully and more exactly. A favoured tool has been grating patterns, which allow a clearly defined measure of resolution acuity, and also of the contrast sensitivity function over a range of spatial frequencies. It rapidly became apparent from this work that resolution acuity gives a very incomplete picture of the visual deficit in amblyopia. Some patients, for instance, show only small losses of resolution but marked loss in contrast sensitivity^{1,2}. Less remarked upon, but also apparent in the data, is that the deficit in acuity for reading the letter chart can be much greater than expected from the deficit in grating resolution. It is tempting to conclude that the contrast sensitivity function is superior to simple resolution acuity as a measure of spatial visual performance.

However, contrast sensitivity is still basically a measure of thresholds of detection, and one of the most striking findings has been that an amblyopic eye is not only impaired in detection, but is also disturbed in how it sees the patterns that it can detect³. Some of this disturbance can be described as a lack of precision in positional information: the bars of a grating, although clearly visible, do not appear in firm and regularly spaced locations. Hess⁴ suggests that we should think of amblyopia as 'scrambled' rather than 'blunted' vision. A measure of this scrambling comes from the work of Lawden⁵, who has shown that amblyopes have difficulty perceiving the relative phase of two grating components, even when they can readily detect that both components are present.

Clinicians have been aware for a long time of a possibly related phenomenon:

amblyopes suffer from 'crowding'. This means that they may be quite good at identifying an isolated letter, but suffer badly from some kind of interference when they have to read letters in a closely spaced row. Again, the simpler acuity task does not properly reflect the disturbance of the patient's vision.

The work reported by Donald Levi and Stanley Klein of the University of Houston in this issue of *Nature* (p.268) shows that many amblyopes suffer an impairment in vernier acuity that is quite out of proportion to their loss of grating resolution. Precise positional information, which the earlier work suggested amblyopes to be lacking, is just what the vernier acuity task demands.

It would be misleading, however, to conclude that amblyopia produces a deficit that is specific to hyperacuity. Levi and Klein's data show that the hyperacuity deficit, while disproportionate to the loss of grating resolution, is quite in line with the patient's deficit in reading the Snellen letter chart. It is the resolution task, demanding only the detection of spatial detail but not information about its spatial configuration, which is the odd man out.

Perhaps more significant than the particular visual tasks involved is the fact that different discrepancies were found in different groups of amblyopes — only the strabismic amblyopes showed a significant loss of acuity. Hess⁶ has already shown a number of differences between strabismic and anisometropic amblyopia, many of which can be summarized by saying that the anisometropes have a uniform loss of sensitivity across the visual field whereas strabismic amblyopia is rather like having normal peripheral vision right across the visual field. There is some evidence that the relatively poor performance on vernier and Snellen acuity, shown by Levi and Klein's strabismic amblyopes, may also be a characteristic of normal peripheral vision.

Amblyopia has also attracted much interest recently because an 'animal model' appears to be provided by deprivation studies of the visual cortex in cat and monkey, beginning with the now classic work of Hubel and Wiesel⁷. A visually deprived eye (including the partial deprivation caused by optical blur) loses its connections to cortical neurones in competition with the eye having normal visual input. The analogy with anisometropic amblyopia is plausible, although the relationship between the degree of connectivity to each eye, which is what physiologists generally report, and the psychophysicists' measures such as acuity, is by no means straightforward. However, the

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animal studies of artificial strabismus⁸ show a loss of cortical cells receiving binocular input, without any evident difference in the degree to which each eye separately signals to the cortex. Such studies provide no obvious basis for amblyopic effects in either eye. Ikeda⁹ has proposed that the deviating eye in strabismus is less often well focused on the subject it is pointing towards, and so suffers a blur quite analogous to anisometropia. The qualitative differences between strabismic and anisotropic amblyopia, revealed by the work of Hess and of Levi and Klein, are difficult to reconcile with this view that they have the same neural basis. On the

other hand, it is still quite unknown what properties of the visual cortex might correspond to the scrambling of the visual world seen through a strabismic amblyopic eye. □

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Crossings and reconnections in superfluid helium-4

from Peter McClintock

AN elegant experiment by F.P. Milliken and K.W. Schwarz (IBM, Yorktown Heights) and C.W. Smith (University of Maine, Orono) has thrown new light on the arcane decay mechanisms of quantized vortex lines in superfluid helium-4. Their work is described in a recent issue of *Physical Review Letters* (48, 1204; 1982).

Below its 'lambda transition' temperature at 2K (-271°C), liquid helium-4 becomes a superfluid and displays a number of highly exotic properties. There is, for example, a component of the liquid which — quite contrary to everyday experience — is capable of indulging in completely frictionless flow and whose viscosity is apparently zero. Correspondingly, an object can move through the stationary liquid without experiencing the

usual resistive drag force. Another closely related property is that the liquid is virtually a superconductor of heat, so that it is extremely difficult to set up a significant temperature difference between two points.

If the velocities of heat fluxes become large enough, however, the superfluid properties disappear abruptly: energy can then be dissipated through the production of quantized vortex lines. These consist of linear singularities, the vortex cores, around which the superfluid circulates at a rate which is quantized in units of h/m_4 where h is Planck's constant and m_4 is the atomic mass. In most cases — for example, if the liquid is made to exceed some critical velocity in flowing through a channel, or where a supercritical heat flux is passed through it — the vortex lines form a random tangle much like a mass of spaghetti, or a hank of steel wool. The situation is a dynamic one, however, because each element of vortex core moves subject to the net velocity flow field due to all the other vortices and also to remote parts of itself, so that the whole tangle is subject to continuous movement and change. A can of writhing worms might therefore be an apter analogy.

The IBM/Orono collaboration has now succeeded in setting up an experimental arrangement which enables them to monitor in remarkable detail what happens to a vortex tangle after the driving force which created it has been removed. The particular feature distinguishing their experiment from numerous earlier investigations, based on flow or heat flow in a channel, is that the measurements were carried out in the bulk of the liquid, remote

from the interfering influence of the walls of the apparatus. The vortex lines were created by high-intensity ultrasound and the density of the tangle, defined as the total length of vortex line per unit volume of helium, was determined from the attenuation of a thin disk of negative ions which could be moved through the tangle by applying an electric field, and which could be stopped momentarily at any chosen position by reducing the electric field to zero for the measuring period of about 0.1 s. Finally, the disk reached a collecting electrode where it gave rise to a pulse of current proportional to the number of free ions still remaining in the disk. Ions are trapped and held by vortex lines at a rate which is quite accurately known as a function of the line density. Thus, the extent to which the disk was depleted during the measuring period could be used to provide an absolute measure of the density of line in the tangle. By stopping the disk at different positions, it was possible to map in some detail the profile of the tangle.

Some experimental results are shown in Fig. 1. The top curve represents the steady-state density profile which was maintained while the ultrasonic transducers were in operation, corresponding to a situation where the vortex growth and decay processes are in balance with each other. The other three curves show how the profile changed with time once the transducers had been switched off. It is interesting to note how little spreading of the tangle occurred with time: the line density decreased rapidly at first, and then more slowly, but with no evident change in the shape of the profile.

A detailed comparison was made between the observed decay of line density and the predictions of Schwarz's microscopic theory (*Phys. Rev. B18*, 245; 1978). It was found that, although the functional form of the decay was in excellent agreement with theory, the absolute value of the decay rate was very considerably

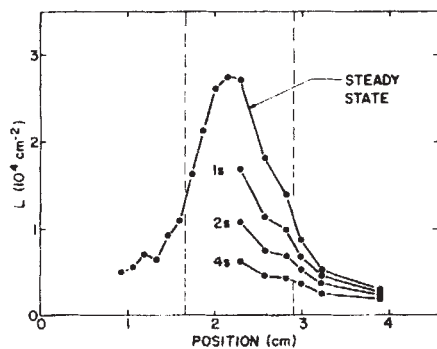


Fig. 1 Vortex line density profile in a chamber of superfluid helium-4 (from F.P. Milliken, K.W. Schwarz & C.W. Smith *Phys. Rev. Lett.* 48, 1204; 1982). The line density L is plotted as a function of position in the experimental cell. The region excited by the ultrasonic transducers is indicated by the dashed lines. The upper curve refers to the equilibrium situation where the transducers are operating; the lower curves show the decaying profile at various times after the transducers have been switched off.

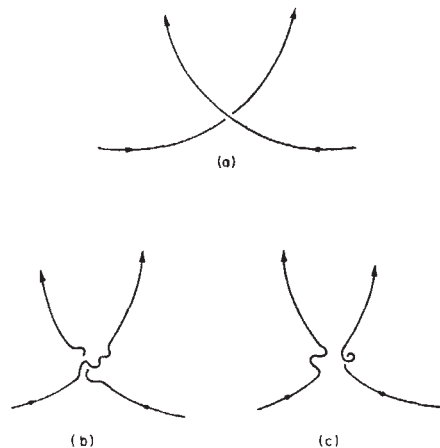


Fig. 2 Two guesses as to what may happen when (a) two vortex lines collide (from K.W. Schwarz *Phys. Rev. B18*, 245; 1978): either they break and reconnect (b) or they break and cross-connect (c).

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smaller than expected. This appears to suggest that the theory is basically correct, but that it requires further refinement. One possibility raised by the authors relates to what may happen when two vortex lines collide with each other at an angle (Fig. 2a). They may cross each other, somehow reconnecting more or less as they were before the collision but with a few local wiggles caused by the mutual interaction of their flow fields, as in Fig. 2b. Alternatively, they may 'topologically cross-connect', as shown in Fig. 2c, so that what was the top end of one line joins to what was the bottom of the other and vice versa. The latter process gives rise to a more substantial curvature of the lines and thus, it can be shown, to a greater rate of line shrinkage, corresponding to a faster net decay of the line density. Thus, one possible interpretation of the present results would be that topological cross-connection processes are much rarer in comparison with line-crossing processes than had been assumed.

In any case, one may hope that further developments in both theory and experiment will now follow, leading eventually to a detailed understanding of the microscopic processes responsible for the decay of quantized vorticity in superfluid helium — something which many people had believed would remain forever a mystery. □

Polymers for solar energy

from Paul D. Calvert

If silicon photovoltaic cells are to become widely used to generate power from sunlight they must become much cheaper to make, more rugged and longer-lived. This requires improvements not only in the photoactive material but also in the encapsulation, the packaging which will protect the silicon from the outside world. It seems sensible to use plastics in the encapsulation rather than expensive and heavy constructions of steel and glass. Unfortunately, many polymers degrade rapidly when exposed to bright sunlight or high temperatures (50°C and upwards), so there are great difficulties in finding suitable materials for solar cells. An American Chemical Society symposium in March brought out these problems. The preprints can be seen in *Polymer Preprints* Vol. 23, no.1.

Much of the work in this area is sponsored by the US Department of Energy, particularly through the 'Low Cost Solar Array' project at the Jet Propulsion Laboratory (JPL). The goal is to produce modules with an efficiency of 10

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per cent and a 20 year lifetime for \$70 m⁻². Of this \$14 m⁻² is allotted to the encapsulant system — a figure greatly exceeded by current systems which use aluminium, glass panels and silicone seals.

A particular problem is the pottant, a soft transparent polymer which serves to surround and protect the brittle silicon cell. Ethylene-vinyl acetate copolymers are favoured but it is doubtful whether they will last for 20 years without discolouration or embrittlement. To test stability conveniently it is necessary to accelerate ageing through the use of high temperatures or intensified natural or artificial light. ARCO Solar Inc. showed with such tests that the pottants degraded where they contacted the cells or the metal connections. Unfortunately, accelerated ageing tests are themselves notoriously unreliable. In the future, more reliable tests might be possible by using highly sensitive spectroscopic methods to follow the slow degradation process under natural conditions early in the life of the polymer. A JPL group is developing laser photo-acoustic spectroscopy while at the Solar Energy Research Institute (SERI) a Fourier-transform IR spectrometer is

Plant biology at the Carnegie Institution

from D.O. Hall

THE *Annual Reports of the Department of Plant Biology of the Carnegie Institution of Washington, University of Stanford, California* make compulsive reading — unlike most annual reports. The latest issue is a most useful summary of recent research and a reminder of how a small, well directed laboratory can produce world-renowned research.

Of particular interest are the reports covering desert plant survival under extreme conditions like those found in Death Valley where the temperature varies daily from 0 to 45°C. Much has been learnt about the photosynthetic carbon paths used, biochemical responses to changing temperature and how components of the membrane enable them to function at high temperatures. For example, *Camissonia* shows the highest photosynthetic capacity recorded to date; an attribute associated with exceptionally high levels of the CO₂-fixing enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco). This attribute is common to a number of plants but, exceptionally, *Camissonia* has a Rubisco enzyme with a higher specific activity; it is thus both more abundant and more efficient.

The various types of stress that interact to limit photosynthetic processes — the damaging effects of extreme tempera-

tures, limitations in CO₂ availability and water stresses, in combination with high and low light intensities — can be described by the catch-all term of photoinhibition. Photoinhibition is well defined as the 'damage to photosynthetic capacity occurring when more light is absorbed by a leaf than can be used by normal photosynthetic reactions . . . it can be induced by any factor restricting utilization of the reducing power generated by light-driven electron transport'. Since photoinhibition is widespread and results in decreased photosynthetic capacity, this pioneering work relates closely to optimizing potential yields from photosynthesis. Specific studies using higher plants and algae and a variety of techniques all point to the conclusion that degradation of the oxygen-evolving reaction (photosystem II) is the main manifestation of photoinhibition under stress regimes. If excess light energy can be dissipated the plant is able to survive.

The ratio of photosystems I to II in the chlorophyll membrane need not be unity, as is often assumed, but can vary depending on the light regime under which the plants are grown. Shady environments or

incandescent lights, for example, both of which have more infrared, result in more photosystem II reaction centres and more granal membranes synthesized. Work is also reported on the role of the chloroplasts of the guard cells of stomata which do not show photosynthetic CO₂ fixation but whose movements are strictly controlled by light. Chloroplasts can now for the first time be isolated from guard cells; they catalyse both photosystems I and II and can make ATP which is consumed when CO₂ is present — by an as yet unknown mechanism.

Finally, the reports from the molecular biology group show how much progress they have made since they were established five years ago. The use of three new pea clones, for example, enables the fine structural organization of repetitive DNA to be documented, which also demonstrates the elegant fine tuning and organizational properties of specific fragments of this DNA. A new emphasis is emerging which will use molecular biological approaches to study the problems identified by physiological research as being so important to plant productivity.

The reports will all probably be published but to have them summarized and documented in detail in a short book is an annual pleasure. Winslow Briggs, the Director, need not be modest in claiming that 'they represent a fine record of high-caliber productivity'. □

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being developed to allow measurements to be made while polymers degrade in the instrument under controlled conditions.

The pottant must be protected from solar UV by an absorbing material on the underside surface. Glass could be used but it is heavy and fragile. A polymer film would be highly suitable but must be very resistant to degradation. Very few clear polymer films will remain tough for long in bright sunlight. Of a great range tested by Boeing in concentrated Arizona sunshine (and for hail resistance with an intriguing iceball throwing machine!) only two fluorocarbons — polyvinyl fluoride and polyvinylidene fluoride (trade names Tedlar and Kynar) — stood up to 10 (accelerated) years without embrittlement or discolouration. Various other points must be kept in mind. The fluorocarbons are not cheap materials and polyvinylfluoride also has the drawback that it is very difficult to process. The stability of the fluorocarbons is thought to be due to the strength of the carbon-fluorine bond but little work has been done on oxidation of these materials and it is not clear how this protects the normally vulnerable C-H bonds in the molecule. Dust tends to stick to the surfaces of polymer films and reduce the transmitted light intensity. Fluorocarbon films, however, seem to be self-cleaning, probably because a surface film forms and washes off with the dust when it rains.

Films are also needed as covers for heated water solar collectors and, when metallized, have been used as 'inflatable' mirrors. There is thus a real requirement for a cheap, transparent photostable film. A group at Brookhaven National Laboratory has designed a solar collector for roof mounting which is made from all thin film materials stretched on a steel frame and estimates that it can thus achieve a tenfold reduction in weight and a fivefold reduction in cost.

As well as passive uses, the symposium provided examples of polymers in the active role, replacing the silicon in photovoltaic cells. Preliminary studies were reported on polymeric phthalocyanines from Xerox of Canada, on heat-treated polyacrylonitrile and on polyacetylene. These materials offer the potential of cheap photocells using powders or thin films as opposed to expensive silicon single crystals. Amorphous silicon already represents a move in this direction. However, these relatively disordered systems are characterized by a high density of defects so that few of the freed electrons manage to travel from the point where light is absorbed to the electrode. Thus pure single crystal silicon reaches efficiencies above ten per cent, amorphous silicon is cheaper to produce but reaches an efficiency of two to three per cent while the organic films are only producing fractions of one per cent in efficiency. Hence the prospect of solar power from polymer painted on the roof of a house is a long way off.

Tree rings and archaeology: dating Roman oaks

from J.M. Fletcher

THE recent discovery of the wooden foundations for a Roman bridge across the Rhine at Koblenz has provided an exciting opportunity for European dendrochronology to prove its worth to archaeology. The dating of the bridge was one of the topics discussed at the recent workshop organized by the European Science Foundation which brought dendrochronologists and archaeologists together at the Wood Biology Institute of the University of Hamburg.

The site of the bridge had been identified when waterway engineers, searching in a large bell for underwater objects which might be a danger to shipping, found stumps protruding from the silt. They appeared to be remains from 600–700 piles which had supported pillars that carried the bridge. For each pillar the Romans had inserted into the river bed, on a regular grid, 25 oak piles estimated to have been 10–15 m long. They were the trunks, about 50 cm wide, of slow-grown trees about 250 years old when felled. From the widths of the rings on stumps extracted from the river bed B. Schmidt (University of Cologne) was able to date construction of the bridge accurately to AD 49 — an achievement possible because of the tree ring chronology built up for central Germany and because some of the stumps retained all their sapwood. Schmidt also concluded that to provide the straightness necessary for being driven into the ground, the oaks were *Quercus petraea* rather than *Q. robur*, and that the level of the Rhine at the time was about 1.5 m higher than at present.

Other applications described at the workshop ranged over several millennia and illustrated the magnitude of the research. Oak, as for the Roman bridge, was the wood used in the numerous Neolithic and Bronze Age settlements by the side of Swiss and other lakes formed as a result of the Ice Age around the Alps. Thousands of samples excavated from more easily available sites are enabling U. Ruoff (University of Zurich) and H. Egger (University of Neuchâtel) to place the settlements in a chronological pattern.

Further north, B. Becker (University of Hohenheim, Stuttgart) and others in Germany have extracted hundreds of oaks from the gravels which mark the ancient course of rivers, such as the Danube, Rhine and Main. The widths of their annual rings have enabled a long oak chronology going back many millennia to be constructed for central Europe. It almost rivals in length the chronology derived from conifers,

including bristlecone pine, which grew in the western part of the US. This European work is of great importance to archaeology for it allows the calibration of radiocarbon results. There has been concern that the American chronology, on which calibration has hitherto been based, may not apply to Europe and other areas. In fact the two calibration curves agree closely in spite of being based on different species, growing in different geographical locations and at different altitudes. Furthermore, by confirming the secular oscillations, or 'wiggles', the European work opens the way to their use as indicators of climatic variations in the distant past.

Another application, to the archaeology of classical, Byzantine and medieval times in the Near East, comes to many as a surprise. Few believed that enough timber had survived in that region to make tree-ring dating a viable technique. In fact, P. Kuriholm (Cornell University), with the enthusiasm and persistence of an explorer, has established chronologies for conifers from Greek and Turkish samples obtained from tombs below ground, and temples and churches above ground. Even the Parthenon and similar temples have contributed to the project, as a small block of wood, known as an *empolion*, was inserted at the interface of each cylindrical 'drum' that built up a column. Small though they were, these pieces often had more than 100 rings of annual growth. The slowness of the growth of the Near East conifers, like that of those in the arid south-west of the US, is a help to the dendrochronologist: a millennium can be covered by relatively few samples while the growth pattern of such slow-grown trees is distinctive and provides reliable matching. As with oaks in north-western Europe, the patterns from trees with narrow rings often match even when grown far apart.

There remains, however, scope for replacing obsolete methodology and for improving the quality of oak chronologies in north-west Europe, since D. Eckstein and S. Wrobel (University of Hamburg) reported that success with dating in the North German plain sometimes amounted to only 50–60 per cent for archaeological samples. This may partly reflect the failure to make use of *weissejahre*, the years in which the change in growth (an increase or decrease in ring-width) is sufficiently consistent among the many samples which form a chronology to act as indicators. These have proved helpful in the more maritime and temperate parts of Europe in matching and thereby dating the fast grown samples of oak, with relatively few annual rings, that form a substantial proportion of building timber.

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Clathrin locks up vesicle structure

from H. Slayter

'COATED VESICLES', present in virtually all nucleated cells, were elevated to the status of 'subcellular particle' in 1975, when a method was derived for isolating these rather heterogeneous (50–150 nm) complex particles. A scarcity of presumed contents following purification notwithstanding, the vesicles appear to be vehicles for the selective transfer of protein molecules into and within cells¹. The vesicles are contained in a 'basket' or 'cage' which proves to be formed from polygonal units — pentagons, hexagons and (rarely) heptagons (Fig. 1). Particles of various size are coated according to the same plan, in which 12 pentagons are interspersed among a variable number of hexagons.

Purification shows that the basketwork from different sources is remarkably constant, consisting of one major protein, clathrin (MW 180,000), together with small amounts of other proteins of molecular weights 100,000 and 30,000. Recently, several groups have looked at — and/or proteolytically tinkered with — purified clathrin and its penchant for reassembly into empty cages. Ungewickell and Branton² observed reversible dissociation

Fig. 1 Large fragments of clathrin cages, negatively stained with uranyl acetate (from Crowther & Pearse *J. Cell Biol.* 91, 793; 1981).

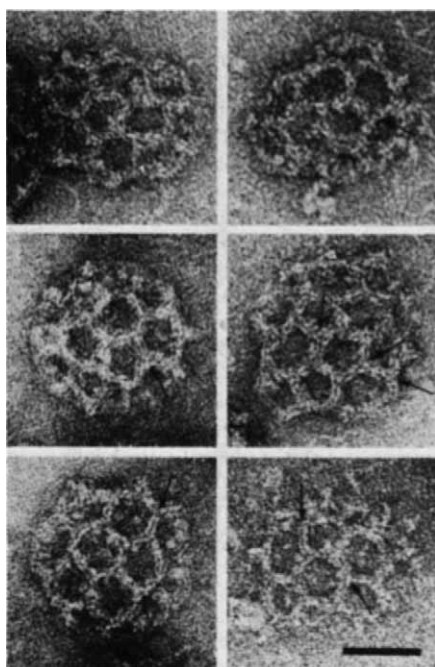


Fig. 2 Electron micrographs of 8.4S clathrin triskelions (from Ungewickell & Branton *Nature* 289, 421; 1981).

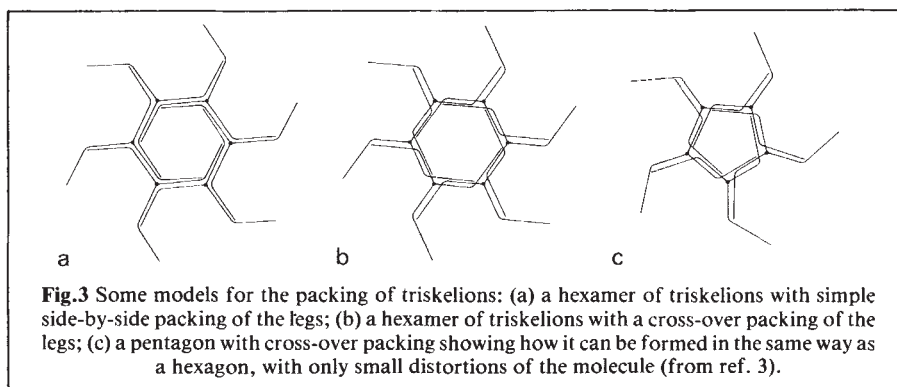
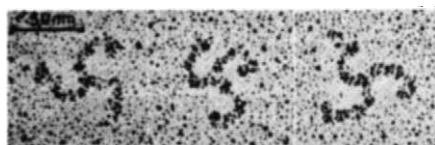


Fig. 3 Some models for the packing of triskelions: (a) a hexamer of triskelions with simple side-by-side packing of the legs; (b) a hexamer of triskelions with a cross-over packing of the legs; (c) a pentagon with cross-over packing showing how it can be formed in the same way as a hexagon, with only small distortions of the molecule (from ref. 3).

of cages into a unit, consisting of three clathrin monomers and three light chains, which they dubbed 'triskelion' (Fig. 2). These 8.4S assemblies include three 445 Å legs, each of which is bent in the same direction at a distance of about 190 Å from a common vertex. [Other authors have since reported leg lengths of 433 Å (ref. 3) and 504 Å (ref. 4) and 160 Å for the proximal segment³.] The triskelions are distinctive, not only in morphology but also as a rare but apparently successful example of molecular weight determination by 'drop counting' of electron micrographs after Williams and Backus⁵. The value of 627,000 so obtained is in remarkably close agreement with the 630,000 obtained by sedimentation equilibrium⁶. Unanue, Ungewickell and Branton⁶ have gone on to demonstrate that triskelions bind with high affinity to uncaged vesicles, via protease-sensitive sites. Meanwhile, Kirchausen and Harrison⁷, using soluble homogeneous 8.65 S clathrin, showed in cross-linking studies that the 8.65 S material is composed of three 180,000-MW chains and three light chains of MW 33,000–36,000, and also that the 8.65 S clathrin can reassemble into cages without the addition of other protein species. The light chain doublet ('clathrin-associated protein' or CAP), which apparently projects from the cage surface⁸, is essential for proper cage formation. After de-CAPPING by chymotrypsin, pentagons and hexagons still form, but fail to join each other.

A recent extension of these studies shows that elastase digestion⁴, even though it does not shorten the legs, prevents the regular handedness of triskelions. It would seem that the CAP, or light chains, serve to glue the triskelion together somewhere near its vertex. Elastase-treated clathrin associates in extended arrays, rather than sealing itself up into tidy cages. Curiously, however, the 110,000-MW major end product of proteolysed clathrin forms cages (despite visible truncation of the

legs), in the absence of light chains, which appear perfectly normal in micrographs.

Crowther and Pearse have applied all the tricks of the trade to obtain the best possible negatively stained electron micrographs of triskelions; that the legs so prepared look 'rather more slender' than those in metal-coated preparations is easily explained in terms of the thickening of the metal coat as well as possible penetration of structures by the negative contract medium. Crowther and Pearse collate various data, proposing a model in which one triskelion is placed at each vertex of the cage (Fig. 3). The inner and outer segments of each leg contribute to two successive sides of a pentagon, leaving the last 80 Å of the clathrin monomer unaccounted for. Successive vertices are slightly rotated, producing cross-over packing of the arms. Thus each polygonal side is apparently composed of two proximal legs from adjacent triskelions together with two distal legs from more distant triskelions. The triskelions evidently are flexible enough to associate with equal ease in pentagonal or hexagonal format, and to form surfaces of various curvatures so as to cover different sizes of vesicle. One wonders whether problems in molecular architecture would be more or less complex if all subunits were as accommodating! □

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Corrigendum

In the report from Leg 84 of the Deep Sea Drilling Project, 'Subduction without accretion: Middle America Trench off Guatemala' (*Nature* 297, 10 June 1982, p.458), we regret that we omitted the name of one of the authors: Roger Helm, from the Institute of Geology, Ruhr University, Bochum, FRG.

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REVIEW ARTICLE

Atmospheric interactions of planetary bodies with the solar wind

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Orbiting spacecraft about Venus have revealed a partially penetrable boundary sheath with induced fields limiting solar wind effects on the atmosphere structure and evolution. Mars differs structurally, the explanation awaiting an orbiter mission such as the European Space Agency is considering. In weak comets gyro-radii exceed other scale lengths, while in active comets the gas and solar plasma couple over the largest scale, boundary sheaths being unimportant—as occurred during early rapid-outgassing phases of Venus and Mars.

WHILE the early flyby missions of Mariners 2, 4 and 5 identified probable bow shocks in the solar wind plasma and showed that Venus¹ and Mars^{2,3} do not possess large magnetospheres, the data were too limited to distinguish unambiguously between magnetic perturbations intrinsic to the solar wind and perturbations generated by interaction with the planets. Interpretation has largely been based on the gas dynamic or magneto-hydrodynamic (MHD) model of single-fluid flow past an impenetrable obstacle⁴, recently refined to cover arbitrary inclination of a convected, weak external magnetic field in three dimensions⁵. It took the Soviet Mars 4–5 orbiting spacecraft to begin to reveal the novel processes and complex physics involved, and NASA's current Pioneer Venus Orbiter with a wide complement of instruments to give a qualitative leap in our understanding.

Before Pioneer Venus, the major characteristics of the solar wind interaction as established by Venera 9 and 10 Orbiters together with flyby and landing craft were as follows⁶. Venus does not have an Earth-like magnetosphere, its magnetic field being largely or wholly the result of induction by the solar wind⁷. The bow shock shape and position are roughly as predicted by gas-dynamic models (standing-off ahead of the planet by 0.15–0.3 times its radius), although there were indications that it is weaker, sometimes closer and more variable than implied by gas dynamics⁸. Such peculiarities together with unusual plasma structures in the wake and lack of energetic particles emanating from the bow shock⁹, led to early suspicions of an extended planetary exosphere contributing a few new ions to the plasma flow and causing some comet-like characteristics¹⁰. Inputs of plasma and energy from the solar wind into the dayside ionosphere as well as into the surprisingly hot and extensive nightside ionosphere had been hypothesized but not directly confirmed¹¹. Viscous-like velocity profiles were observed to extend downstream from the terminator, with streaming plasma bending into the planet wake¹². Such profiles are expected when ion gyro-radii are comparable to the ionosheath thickness¹³, but planetary ions drawn out into a comet-like tail also provide a plausible explanation¹⁰.

How far the Mars 2–5 probes revealed differences between the Mars and Venus interactions is still controversial and likely to remain so until a further Orbiter is sent to Mars. As at Venus, an ionosheath extending into the tail constitutes a gradual transition from shocked solar wind to slowly-moving planetary ions^{14,15}. The velocity profiles on the flank resemble shearing flow rather than the canonical gas-dynamic model with a tangential discontinuity⁴. If the transverse scale of some 1,000 km corresponds to gyro-radius, the heavy ions are inferred to be C⁺, N⁺

and/or O⁺, but there are questions over the detector and close identification is still lacking. Unlike at Venus where the ionosheath converges rapidly into the tail and perhaps emits a rarefaction wave, the diverging martian sheath is relatively limited (0.5 radii at 3–4 radii downstream) and has a rarefied interior containing little inhomogeneous plasma^{14,15}. However, the extensive tail region and the nightside ionosphere have been explored rather little. The Mars bow shock stands further ahead of the planetary surface (0.5 rather than 0.3 ± 0.07 radii, according to new and compatible fitting procedures¹⁶) and the sheath has a wider flaring angle (some 30° compared with 20°)^{17,18}. The low ionosphere density, presence of suprathermal ions ahead of Mars and abnormally cool ions in the sheath, and deviations from the gas dynamic model led to an early challenge to the magnetosphere idea¹⁹. This was reinforced by a demonstration that the magnetic field direction is consistent with solar wind field 'draped' around the planet²⁰. Unfortunately the Viking Lander failed to carry even a simple magnetometer to resolve the issue of intrinsic Martian magnetization.

Three extreme models of the solar wind–atmosphere interaction have been proposed^{21,22}, as shown in Fig. 1. In model *a*, a strong magnetic field is supposed to be induced in the ionosphere, whose pressure counterbalances the solar wind's dynamic pressure. The configuration can be termed a 'pseudo-magnetosphere' by analogy with the Earth's magnetosphere. In model *b*, the magnetic field is assumed to remain weak, while ionospheric pressure balances the solar wind. In model *c*, the solar wind is pictured as flowing steadily into the atmosphere, being continuously modified by ionizing interactions. The plasma is eventually absorbed, through recombinations in the lower ionosphere. While *c* is perhaps appropriate to comets, it was recognized¹⁹ that the steeply increasing density in the Mars

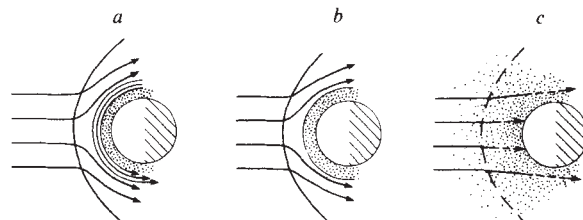


Fig. 1 Interaction models: the solar wind may be held off: *a*, by magnetic pressure; *b*, by ionospheric pressure; or *c*, partially absorbed.

and Venus atmospheres and their small amounts of H and He render invalid the absorption plus weak shock model. However, the need to take into account the comet-like plasma interaction with neutral atmosphere still remained^{19,22}.

Venus interaction

The Pioneer Venus Orbiter marks a new stage in investigations of the atmosphere interaction with the solar wind, in giving us far greater detail over an extensive region of the planet for a prolonged period. Special journal issues assemble many of the experimental results²³. Pioneer Venus established that the bow shock is indeed different from the Earth's, being weaker and closer to the obstacle²⁴, and probably departing from cylindrical symmetry^{25,26}. Its position is insensitive to solar wind parameters and its limbs do not approach the Mach cone angle²⁷. The shock structure is more diffuse, and is associated with enhanced plasma waves at 5 and 30 kHz, presumed due to planetary ions and consequent collective oscillations²⁸. Pioneer Venus Orbiter data confirmed the induction concept⁷, showing that currents and fields are induced within the ionosheath so as generally to exclude solar wind plasma and field²⁹, this sheath transition being a few tens of kilometres thick, at an altitude of typically 350 km at the nose increasing to 1,000 km at the terminator^{30,31}. Figure 2a shows plasma and magnetic parameters along an orbit through the sheath into a low-field region

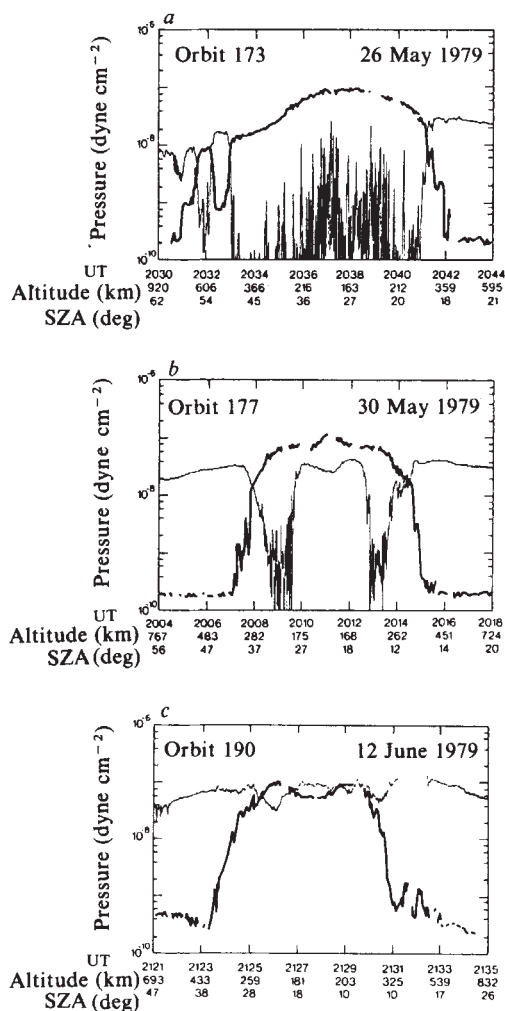


Fig. 2 Profiles from Pioneer Venus Orbiter³² of magnetic (thin lines) and plasma (thick lines) pressures near periapsis, assuming electron temperature equal to that of the ions: *a*, the 'field-free' ionosphere with sporadic 'flux rope' enhancements; *b* and *c* magnetized ionosphere configurations. Note that the two pressures tend to have anti-correlated fluctuations. Probably $T_e = 2-3 T_i$, so the plasma pressure could be larger than shown here^{25,26}.

and out again³². Note, however, the spiked enhancements of magnetic field outside the ionosphere, which correspond to twisted 'flux-rope' structures that occupy some 5% of the volume traversed³³. The peak fields in the sheath of a few times 10^{-4} G exert the main pressure counterbalancing the solar wind, while the plasma pressure contributes some 20–30%. During periods of enhanced solar wind, the observations³² stood in strong contrast (Fig. 2*b, c*). Apparently the ionosphere responds diamagnetically, resisting compression through electromagnetic induction of currents and a strong ionospheric field. This is understandable if the main induced field adjusts to solar wind changes with a finite delay time of 30 min or less⁶, consistent with a conductivity of $1-2 \text{ S m}^{-1}$ and so similar to estimates from gas-plasma collision rates¹⁸. At quiet times the field structure outside the sheath generally corresponds to solar wind field lines draped around the planet, with a corresponding ionospheric current system³⁴, but there seem to be connections to the ionospheric field and some interchange of ionospheric and solar wind plasma. Various possibilities have been identified: energetic solar wind may enter the dayside ionosphere along the twisted flux ropes of enhanced field^{29,31}, and some ionosheath solar wind may flow along field lines penetrating the nightside ionosphere, though flow from the upper dayside ionosphere seems to be the major ion source³⁵. Fluctuating magnetic fields permit a fraction of the solar wind protons to diffuse into the terminator region³⁶, but dissipation of the induced currents is favoured for an external energy source on the dayside²⁹. Figure 3 illustrates the large scale structure^{6,34} and indicates the general plasma characteristics. Non-steady behaviour of the flow is important, perhaps dominant. Clouds of plasma appear to be stripped off the ionosphere in response to increases in the solar wind dynamic pressure^{30,31}; these clouds could be the source of O^+ ions convected far downstream.

On the other hand, ionospheric chemistry implies that a weak suprathermal atomic O component of some 10^4 atoms cm^{-3} extends far out in the exosphere, to 3,000–5,000 km altitude³⁷. When ionized in the solar wind, the oxygen constitutes additions of mass to the flow³⁸, it has been hypothesized, causing sheared velocity profiles and an apparently blunter obstacle. This magnetospheric rather than spherical-ionospheric shape has been modelled with an unphysically hot ionosphere^{4,5}. Moreover, the new ions' gyro-radii of around 1,000 km exceed the ionosheath and ionosphere scales, so some of them should precipitate into the ionosphere and appear comparable with the energetic O^+ associated with substorms on Earth³⁸⁻⁴⁰. The dependence of precipitation on magnetic field direction could explain the observed shock asymmetry^{8,25}. The relative importance of continuous sweeping up of new ions compared with detachment of ionosphere clouds is still unclear. Whichever the case, the general conclusion from Pioneer observations is that the interaction with Venus is less influenced by the degree of absorption of plasma than by planetary ions injected into the flow—influenced by comet-like sources of plasma rather than 'sinks'⁴¹.

Mars interaction

Despite the many similarities indicated above, Mars does seem to differ significantly from Venus in its more distant bow shock, its wider-flaring, open ionotail^{14,15}, but a less extensive ionosphere on the planet flanks¹⁸. The magnetometer and electron-probe experimenters argue¹⁷ that these differences imply a magnetic field intrinsic to Mars of dipole moment $2.5 \times 10^{22} \text{ G cm}^3$. Independent examination of the published magnetic data has caused Russell²⁰ to dispute this: he set the dipole moment smaller by at least a factor 10 and argued that the magnetic field direction generally agrees with solar wind fields 'draped' over the ionosphere plus induced fields in the ionomagnetsheath. The larger value gives just enough magnetic pressure to hold off the quiet-time solar wind above the planet atmosphere—but not enough to withstand enhanced solar wind fluxes. A dynamic Venus-like interaction, with most of the field

Table 1 Solar wind parameters based on ref. 14 and derived interaction length scales (equation (3))

	AU	P_{stag}	Mach no.	Magnetic B (nT)	Spiral angle	$4\pi Q$ (s^{-1})	Length scales (km)			
							R_B	R_{coll}	R_*	R_i
Venus	0.72	5.0	6.6	10	36°	10^{27}	6,050	—	6,410	2,000
Mars	1.52	1.1	7.9	3.3	57°	10^{27}	3,500	—	3,800	3,000
Comet Encke }	1.0	2.5	7.2	6	45°	10^{27}	1	30	1,000	2,500
Comet Halley }						10^{29}	3	3,000	10^5	2,500

Units of the ram pressure P_{stag} are $10^{-8} \text{ dyn cm}^{-2}$. Comets Encke and Halley are taken as typical examples of weak and active comets respectively at 1 AU, but all their parameters change with heliocentric distance.

being induced, is implied during periods of enhancements if not also during quiet conditions.

An intermediate stance on the dipole strength is taken by Intriligator and Smith⁴², on the basis of the magnetic pressure necessary to supplement the plasma pressure of a model ionosphere. Figure 4a shows their magnetosphere model for a dipole moment $8 \times 10^{21} \text{ G cm}^3$ (10^{-4} times the Earth's, giving an equatorial surface field of 21 nT), comprising two novel features: (1) the polar caps cover a significant portion of the planet's surface, and (2) the nightside atmosphere is directly connected to a tailward plasma-sheet. Planetwards convection of wake plasma at all longitudes is expected^{43,44} (Fig. 4b) because the solar wind-induced electric field ($-\mathbf{U}_{\text{sw}} \times \mathbf{B}_{\text{sw}} \sim 1 \text{ kV}$ across the diameter) is an order of magnitude larger than a co-rotation electric field at the magnetopause ($R_{\text{MP}} \Omega \times \mathbf{B}_{\text{MP}}$). Ion-atmosphere collisions define a lower limit for the penetration of such convected plasma flow. Although the large polar caps appear open to the solar wind (Fig. 4a), it is possible that induction acts on the Venus pattern so as generally to impede penetration into the ionosphere. On this model the Mars interaction would be sensitive to the solar wind field orientation: because of the tendency to 'field reconnection', the front-side magnetosphere would tend like at Earth to be unstable when the solar wind and planetary fields are anti-parallel, but be relatively stable when parallel.

Alternative explanations have been proposed for the Mars shock and ionosheath lying further out than for Venus^{18,45} (Fig. 2). Its exosphere has different composition and is more extensive, its ionosphere has higher conductivity, the planet is a relatively rapid rotator, and the solar wind β (ratio of flow to magnetic energy) is smaller at Mars. The latter two points are not supported as explanations by scaling laws^{42,43}, collisional conductivities are lower at Mars rather than higher¹⁸, and in the light of Pioneer Venus observations, the exospheric explanation looks the most promising. The suprathermal O input from O_2^+ recombination is comparable on the two planets⁴⁵ but the martian exosphere is more extensive because of lower gravity. Suprathermal atomic N from various chemical processes provides an additional exospheric component of both escaping and bound gas⁴⁶.

Comets

Both Mars and Venus are believed to have lost much of their primitive atmospheres during their evolution. Instead of provoking minor modifications to the solar wind flow, the abundant escaping exosphere at earlier epochs would have given them the character of comets. It is now understood that ions created in the extensive comas of larger comets dominate the oncoming solar wind⁴⁷. Virtually all the solar protons that penetrate to a few times 10^4 km are neutralized through charge exchange⁴⁸. Theory indicates that new cometary ions would be picked-up by large scale **E** and **B** fields or by fluctuating small scale fields, the latter resulting from plasma instabilities driven by the relatively streaming ion beams^{49,50}.

In comets where new ions have relatively small gyro-radii, their primary effect appears as 'mass loading' giving deceleration of the solar wind flow^{47,51}. In a supersonic one-dimensional flow, the maximum proportional mass addition is $(\gamma^2 - 1)^{-1}$. For a source flow of strength Q molecules $\text{s}^{-1} \text{ sr}^{-1}$

at speed V interacting through charge exchange with cross-section σ_{ex} , the deceleration radius is given⁵² by

$$(\gamma^2 - 1)^{-1} = A_i \int_{R_*}^{\infty} \sigma_{\text{ex}} \frac{Q}{V} \frac{dR}{R^2} \quad \text{or} \quad R_* = (\gamma^2 - 1) A_i \sigma_{\text{ex}} \frac{Q}{V} \quad (1)$$

taking the density to follow an inverse square law. (Models in which the density drops off much more steeply with R , due to radiation pressure⁵³ or to plasma drag⁵⁴, are unrealistic.) With mean atomic weight $A_i \sim 20$ and $Q/V \sim 10^{23} \text{ cm}^{-1}$, this gives an estimate of $R_* = 10^5 \text{ km}$ for a good-sized comet such as Halley. Allowance for photo-ionization and more readily ionized components such as OH gives a slightly larger estimate for R_* , but because photo-ionization makes a minor contribution the value depends little on solar fluxes⁵². This parameter R_* is the scale standoff distance for the cometary bow shock, weakened because the plasma ahead of it is energized through the pick-up processes: on model calculations its Mach number is ~ 2 (refs 51, 55, 56), even weaker than that observed at Venus. If instability-associated processes are sufficiently dissipative, the cometary shock as a region of enhanced dissipation may even be absent.

The scale for plasma-gas coupling and limit of the collision-dominated coma

$$R_{\text{coll}} = \sigma_{\text{coll}} Q / V \quad (2)$$

is in all cases smaller than R_* by more than a factor 10 because the cross-section factors are comparable. The shock stand-off distance R_* is thus far greater than (and unrelated to) the effective obstacle size, unlike normal hypersonic flow past an obstacle such as a magnetosphere⁵¹. The flow regions deduced are depicted in Fig. 5a.

Such a novel shock has not yet been investigated in practice and several points in the argument need experimental

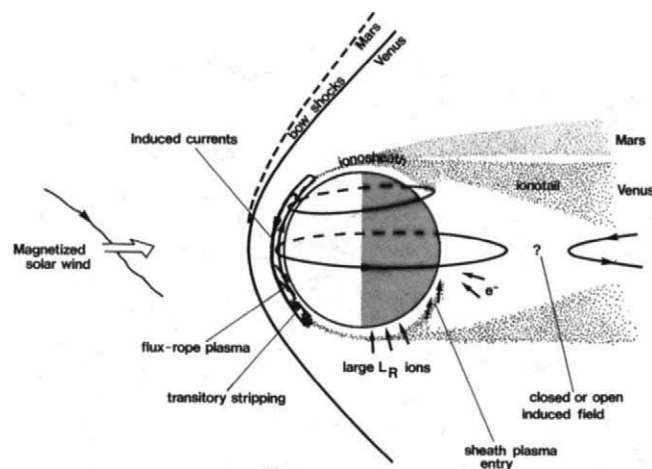


Fig. 3 Schematic of the Venus interaction with the solar wind, showing large-scale induced current and magnetic structure, and indicating various mechanisms for plasma interchange identified by Pioneer Venus. Representative positions of the wider bow shock and ionosheath at Mars as detected by the Mars Orbiters are shown in comparison (scaled to the planet radius).

verification. The net result of the ion pick-up interaction can differ significantly from Venus in the way the energy of the decelerated solar wind is distributed between plasma species and fields. A weak resistive shock causes little heating of the ions and electrons (order 10 eV per particle) while pick-up in a transverse field can give up to keV per nucleon to cometary ions in the outer coma¹⁹. In practice the fraction of energy going to the protons and electrons is probably not negligible but still relatively small.

Cometary ions picked up in the far unperturbed solar wind have gyro-velocities up to the solar wind speed and so gyro-radii R_i of the order of 10^4 km. Thus for weaker comets such as Encke with²⁷ peak $Q/V \approx 10^{21}$ H₂O molecules cm⁻¹, equation (1) gives $R_* < R_i$. However, R_* still exceeds the obstacle size R_{coll} from equation (2) and the body radius R_B (comet nucleus radius 1–5 km). The collisionless shock thickness R_s is a few solar wind proton gyro-radii, that is $R_s \approx (0.1-1)R_i$. At comet Encke's peak with $R_* \approx R_i$, the fluid description with ion pick-up is scarcely valid, and further from the Sun where Q drops by a factor of 10–100, the scale parameters characterizing a weak comet are ordered as

$$R_i \gg R_s > R_* > R_{\text{coll}}, R_B \quad (3)$$

Comet Halley outside 3 AU is similarly weak. The cometary ions do not see the tiny comet nucleus or collisional atmosphere, and the potential disturbance scale R_* is smaller than the shock thickness. The dominant physical process is still interaction with large gyro-radius cometary ions, but as individual charged particles rather than a quasi-fluid. Laboratory experiments⁵⁸ also have $R_i \gg R_* > R_B$, so they simulate weak comets (but not typical ones). For Venus, Mars and large satellites such as the Moon, $R_B \gg R_i$ unlike relationship (3), so special ionosheath or boundary structures are plausible for them but not expected for comets.

Dust grains up to 30 μm radius can be blown off during comet Encke's maximal outgassing (rate $10^{26} \text{ s}^{-1} \text{ sr}^{-1}$), while at lower rates, spin of the nucleus or electrostatic forces⁵⁹ may help expel the grains. Micrometre-sized grains attain terminal speeds

of 10 m s^{-1} , so are blown tailwards by radiation pressure on the 100-km scale⁶⁰ (since speeds $\sim Q^{1/2}$, the scale is of order $0.1R_*$ for all Q at 1 AU, of order R_* at 3 AU). The dust coma and tail consist of individual grains, with only the finest ones ($a < 0.03 \mu\text{m}$) which see little radiation pressure⁶⁰ able to penetrate far laterally. Such grains being charged suffer perturbations from the solar wind fields as well as sputtering by solar protons, but on far larger scale lengths. Their own effect on the plasma flow is negligible, proton collisions with dust grains being much less frequent than with neutral gas, supposing gas and sub-micrometre dust production at similar rates and a velocity ratio of order 100. Thus for a weak and dusty comet we derive the situation sketched in Fig. 5b, with the dust and plasma essentially uncoupled. Some long-lived large (1 mm–1 cm) grains⁶¹ can also extend past the main dust envelope (Fig. 5b), possibly because the nucleus spin is rapid enough to eject grains against gravity.

The famous plasma envelopes are probably distinctive of large comets, being dependent on small gyro-radius. The CO⁺-rich Comet Morehouse of 1908 showed a series of plasma envelopes forming at a distance of $1-3 \times 10^5$ km ahead of the nucleus^{62,63}. The time scale for formation was typically 1 h or so; as each envelope grew, it seemed to recede towards the nucleus. In the course of contraction, the structures became sharper and sometimes connections could be traced between these envelopes and the symmetric ion rays in the tail. Morphologically speaking, these symmetric tail rays may reflect inhomogeneous ion production, with ions expanding along field lines, or the collapsing of denser ion shells extending from the envelopes, like a folding umbrella⁶⁴. Either way these features are indicative of the non-stationary and inhomogeneous nature of the solar wind-comet interaction.

The region inside R_{coll} (equation (2)) where the ions and electrons must be closely coupled to the outflowing cometary gas, is termed the cometary ionosphere (Fig. 5a). It differs from a planet's ionosphere in that the dynamical expansion interferes with photochemical equilibrium, the composition changing with radius R . However, computations^{65,66} show that the overall ion density and stagnation pressure are not far from the photochemical law: $p_{\text{stag}} = p_e + p_i + \rho_i V_i^2 \propto R^{-k}$, $k \approx 1$. The transition from the ionosphere to the plasma inflow (solar wind plus picked-up cometary ions) is still a matter of controversy^{48,54}. Downstream of the shock, collisional cooling processes compete with ion pick-up deceleration, but the pressure in the subsonic region is expected to stay not much below the solar wind stagnation pressure $P_{\text{stag}} = p_\infty + \rho_\infty U^2 + B^2/8\pi$ (refs 51, 56). The ionospheric p_{stag} might exceed this if heating is high or ionization processes are enhanced; but probably collisional cooling and recombination keep $p_{\text{stag}}(R_{\text{coll}}) < P_{\text{stag}}$ by more than a factor of 10 (ref. 48). In this situation, the stagnation flow occurs well into the collisional region and interaction with the neutral gas also dominates the incoming plasma flow. Inclusion of magnetic field whose pressure is limited to around P_{stag} would not change the situation qualitatively⁵⁶.

Among cometary ions, CO⁺ usually has the strongest optical emissions. CH⁺, N₂⁺, CO₂⁺ and H₂O⁺ are also evident around 10^4 km from the nucleus and extending into the tail⁶⁷; H⁺, C⁺, O⁺ and OH⁺ should be more numerous and extensive, but still await direct detection. Much information is contained in the morphology of the ion tails long observed from Earth. The generation and gradual folding of the CO⁺ ion rays onto the central tail axis are doubtless indicative of magnetic field 'draping'⁶⁸, the sheared velocity field of the plasma flow, a dynamic solar wind interaction and/or inhomogeneous ion production mechanisms^{69,70}. Large-scale wave structures seen in some tails are well modelled as a non-linearly stabilized Kelvin-Helmholtz helical mode⁷¹.

By analogy with the magnetotails of Earth and Venus, the presence of a current sheet (or sheets) and reconnection processes can be expected⁷². Ionization through auroral-type electrons and plasma acceleration through Lorentz forces have been hypothesized⁷²⁻⁷⁴. Accelerating potentials presumably depend

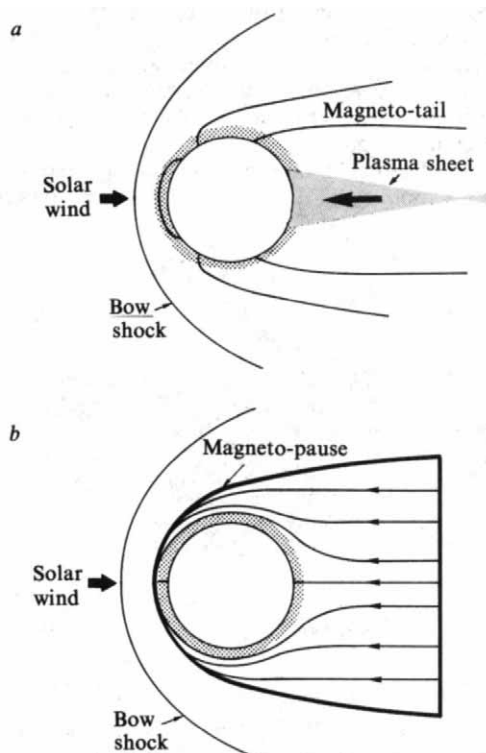


Fig. 4 The hybrid martian magnetosphere for a marginally important intrinsic planetary field (after ref. 42) in planes normal (a) and parallel (b) to the dipole.

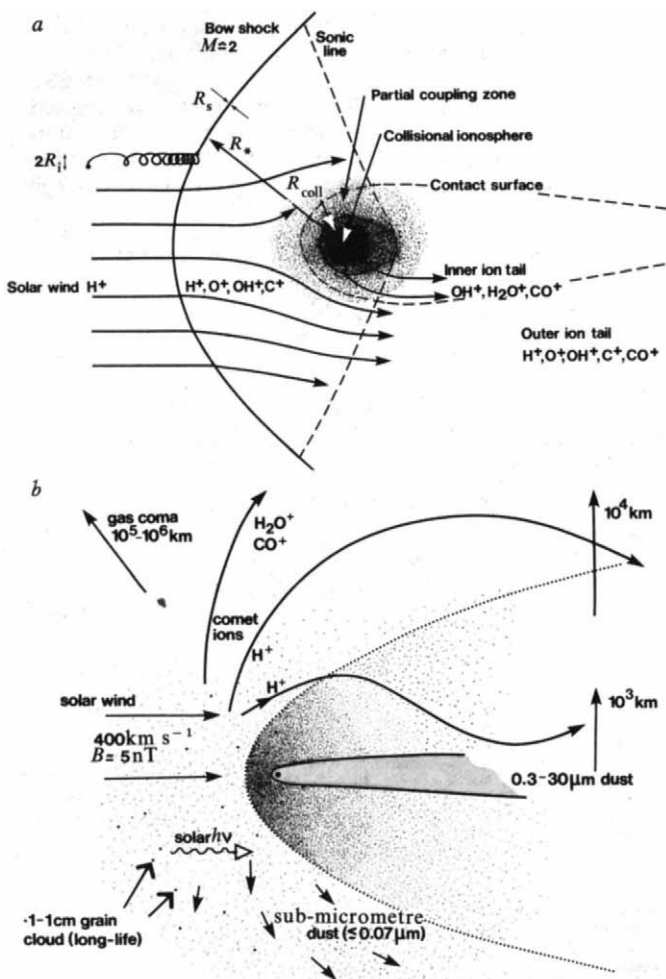


Fig. 5 Flow regions and scales: *a*, in a larger, gassy comet with streamlines indicating the mean plasma flow, though individual ions may undergo chemical reactions before progressing far along them; *b*, in a weak dusty comet where ion gyroscs and molecule free paths are large. Micrometre-sized dust released with the gas behaves as individual grains in a largely undisturbed solar wind, whose low speeds mean that radiation pressure blows them back into a narrow tail. Long-lived grains and sub-micrometre dust, (small enough to avoid most photons) populate the outer dust coma.

on the permeability of the ionosheath and the potential of the motional electric field across the tail $\phi = UBd/c$ for the tail diameter d . Although the values of d for comets and Venus are more than an order of magnitude smaller than the Earth's, the permeability of thick cometary ionosheaths is probably less than the unstable Venus one, so accelerating potentials would be intermediate at several kilovolts. Mean fields in the comet tail have strength similar to the interplanetary B_0 and as at Venus can have dynamically a near-passive role. However, higher fields up to 50 nT corresponding to p_{stag} should exist in the stagnation region²⁹ and perhaps in inhomogeneous structures analogous to the Venus flux ropes³³.

Conclusions

A realistic model of the Mars and Venus interactions with the solar wind must contain elements from each of the extreme cases depicted in Fig. 1. The induced field and ionospheric plasma in combination counterbalance the solar wind, while neutral atmosphere outside the ionosphere interacts with the plasma. Additional elements demonstrated by Pioneer Venus are the non-stationary nature and a degree of permeability of the ionopause to plasma and small scale fields^{30,31}. The ionosphere's response, still largely unknown, is presumably charac-

terized by the strong variability revealed in the ionosheath thickness and position^{30,31}, in some ways analogous to cometary 'envelopes' and tail rays. The Mars situation may be complicated by a small planetary field, making it a hybrid of Mercury and Venus⁴² and more unstable when the interplanetary field is anti-parallel. Such a field is too weak to hold off the solar wind at all times¹⁸, so Mars constitutes a novel transitional case of solar wind interaction. The planet's high rotation rate and atmosphere transparency imply that latitudinal variations and ionospheric winds will be much less severe than at Venus. One lesson from Pioneer Venus is that plasma analysers covering the full mass-energy range from solar wind protons to ionospheric O^+ , O^{2+} , CO_2^+ at thermal energies are clearly needed. A second is that high time resolution is essential and dual spacecraft studies greatly advantageous for analysing the moving structures.

From a scientific point of view, Pioneer Venus opened up a whole spectrum of interesting physics: a Mars orbiter mission as is under study by ESA is the natural sequel. Not only are we now clearer on what to expect, we also know the kind of instrumentation needed. The novel phenomena discovered by the Pioneer Orbiter as well as the striking differences confirmed in the plasma and atmospheric environments of the two planets require systematic study. Specification of the non-thermal planetary ions should reveal the acceleration processes: a combination of ion sensors with magnetometer and plasma wave detectors would distinguish the role of adiabatic and plasma-turbulent pick-up of ions from the outer exosphere. The important non-thermal neutrals resulting from charge exchange, dissociative recombination, solar wind sputtering and energetic ion precipitation are difficult to measure, but new instruments are under development. Direct exploration of the hot corona of suprathermal neutrals (barely detected by the Venera and Pioneer spacecraft) may thus be feasible.

The current atmosphere loss from Venus and Mars is determined not by thermal processes but by the solar wind interaction and ionospheric chemistry. Calculations of the Venus H-loss consistent with explanations for the extensive corona observed in H Ly α , range from $8 \times 10^6 \text{ cm}^{-2} \text{ s}^{-1}$ up to $1 \times 10^8 \text{ cm}^{-2} \text{ s}^{-1}$, the latter for a maximum hypothetical H_2 atmospheric component^{75,76}. Ionization of the suprathermal O above the ionopause^{37,77,78} averages $1 \times 10^7 \text{ cm}^{-2} \text{ s}^{-1}$ (mean ionopause heights of ref. 30) interaction with the solar plasma may return 25% to the planet and sweep the remainder into the tail (this flux is a factor 2-3 smaller than that estimated for O^+ structures convected into the wake³¹). It is not yet clear whether the H and O losses are in the ratio of H_2O , as indicated for Mars, or whether atmospheric O is absorbed in Venus surface rocks.

The equivalent planetary interaction parameters to those for weak and active comets are estimated as in Table 1. The plasma-atmosphere coupling radius R_* for Venus and Mars is determined not strictly by equation (1) but as the radius at which a tangential solar plasma flow suffers substantial deceleration²². That the O^+ gyroradii are large undermines such a concept, but with the steeply-varying exosphere density the uncertainty in R_* is small. Comparison of the radial scales in Table 1 shows the different ordering of R_i and R_* for the two comets, as well as their similarity to the body size R_B for the planets; the latter implies that boundary layer and finite gyroradius effects are significant.

It is known⁷⁹ that Mars through its history has lost much of its N and O: the 64% enrichment of the heavy ^{15}N isotope recorded by Viking⁷⁹ is explained by chemical processes producing atoms above the escape speed. In contrast the negligible enrichment of the ^{18}O isotope suggests that solar wind pickup with no isotope preference has overwhelmingly dominated the O-loss processes. At earlier evolutionary epochs Mars and Venus both had far more H_2O and loss rates were doubtless far higher^{80,81}. The 'runaway greenhouse' effect is invoked⁸⁰ to explain Venus loss rates 10^6 times higher than present. This implies a total loss $4\pi Q \sim 3 \times 10^{31} \text{ H}_2\text{O s}^{-1}$, a factor 10^2 - 10^3 larger than the model for comet Halley (Table 1). Equation (1)

implies an interaction scale with the solar wind of $R_* = 10^7$ – 10^8 km, a truly giant comet. (This extrapolated scale should not be taken literally, as photoionization and radiation pressure restrict the extension into space of the neutral gases.)

Clearly, intermediate stages during Mars and Venus atmosphere loss would also be cometary. Justification for the Giotto mission to Comet Halley and for a new Mars Orbiter mission lies not merely in winning understanding of their present inter-

actions with the solar wind, but also in achieving realistic modelling of the planets' past and future evolution.

An initial version of this review was presented at the ESF Workshop on Planetology at Strasbourg (1980) and revised while M.K.W. was a visiting scientist at the Max-Planck Institut für Aeronomie, Lindau, and holding an SRC Fellowship at the Mullard Space Science Laboratory of University College London.

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ARTICLES

Geology and chemistry of hydrothermal deposits from active submarine volcano Loihi, Hawaii

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High-resolution bathymetric surveys, bottom photography and sample analyses show that Loihi Seamount at the southernmost extent of the Hawaiian 'hotspot' is an active, young submarine volcano that is probably the site of an emerging Hawaiian island. Hydrothermal deposits sampled from the active summit rift system were probably formed by precipitation from cooling vent fluids or during cooling and oxidation of high-temperature polymetallic sulphide assemblages. No exotic benthic fauna were found to be associated with the presently active hydrothermal vents mapped.

LOIHI Seamount is an active submarine volcano that marks the southernmost extent of the Hawaiian 'hotspot' and the probable site of an emerging Hawaiian island (Fig. 1). It is one of the two known active hotspot submarine volcanoes of the

Pacific Ocean; the other is the relatively inaccessible Macdonald Seamount of the Austral Island Chain¹. Although Loihi is ~50 km south-southwest of Kilauea caldera, it apparently is not volcanically associated with Kilauea's south-west rift zone².

Table 1 Major and minor element chemistry and atomic proportions of Loihi Seamount hydrothermal deposits

Wt %	Loihi no. 1 bulk	Loihi no. 2 > 44 μm	Loihi no. 2 < 44 μm	Loihi no. 2 < 44 μm CBD treated	DSDP Leg 54, hole 424A-1-1, 50–59 cm < 2 μm , CBD treated	DSDP Leg 54, hole 424B-2-4, 74–82 cm < 2 μm , CBD treated
Na ₂ O	0.5 $\pm$ 0.2*	0.04 $\pm$ 0.01	0.07 $\pm$ 0.03	0.2 $\pm$ 0.05	0.3 $\pm$ 0.2	0.2 $\pm$ 0.05
K ₂ O	0.4 $\pm$ 0.1	1.1 $\pm$ 0.3	0.4 $\pm$ 0.1	0.6 $\pm$ 0.05	1.3 $\pm$ 0.1	2.1 $\pm$ 0.05
CaO	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	0.6 $\pm$ 0.4	0.7 $\pm$ 0.1	0.8 $\pm$ 0.2	0.32 $\pm$ 0.03
MgO	1.5 $\pm$ 0.2	2.9 $\pm$ 0.2	3.4 $\pm$ 0.6	2.3 $\pm$ 0.2	3.6 $\pm$ 0.2	3.0 $\pm$ 0.1
Al ₂ O ₃	1.5 $\pm$ 0.1	0.9 $\pm$ 0.4	2.5 $\pm$ 0.4	2.4 $\pm$ 0.2	4.4 $\pm$ 1.0	0.7 $\pm$ 0.1
SiO ₂	19.8 $\pm$ 3.7	50.1 $\pm$ 2.0	39.9 $\pm$ 2.9	50.2 $\pm$ 3.0	51.3 $\pm$ 1.3	52.4 $\pm$ 0.3
FeO _T	61.0 $\pm$ 6.7	29.7 $\pm$ 2.0	38.0 $\pm$ 1.7	28.7 $\pm$ 1.8	22.5 $\pm$ 1.1	26.5 $\pm$ 1.2
MnO	0.06 $\pm$ 0.05	0.03 $\pm$ 0.2	0.1 $\pm$ 0.1	0.02 $\pm$ 0.02	0.6 $\pm$ 0.2	0.07 $\pm$ 0.06
TiO ₂	0.06 $\pm$ 0.02	0.02 $\pm$ 0.02	0.4 $\pm$ 0.2	0.3 $\pm$ 0.05	0.5 $\pm$ 0.7	0.03 $\pm$ 0.01
Cr ₂ O ₃	0.02 $\pm$ 0.03	0.02 $\pm$ 0.02	0.00 $\pm$ 0.00	0.03 $\pm$ 0.03	0.00 $\pm$ 0.00	0.01 $\pm$ 0.02
Total	85.3 $\pm$ 4.6†					

	Loihi no. 1 bulk§	Loihi no. 2 > 44 μm	Loihi no. 2 < 44 μm	Loihi no. 2 < 44 μm §	Loihi no. 2 < 44 μm , CBD	DSDP54-424A- 1-1, 50–59 cm < 2 μm , CBD	DSDP54-424B- 2-4, 74–82 cm < 2 μm , CBD
Tetrahedral layer							
Si ⁴⁺	7.26	7.62	6.33	7.34	7.58	7.67	7.92
Al ³⁺	0.64	0.17	0.48	0.55	0.42	0.33	0.08
Fe ³⁺	0.10	0.21	1.19	0.11			
	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Octahedral layer							
Fe ³⁺	3.37	3.56	3.86	3.40	3.62	2.81	3.34
Al ³⁺						0.44	0.04
Ti ⁴⁺	0.02		0.04	0.05	0.03	0.06	
Mg ²⁺	0.61	0.44	0.10	0.55	0.35	0.69	0.62
	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Interlayer							
Ca ²⁺	0.16	0.06	0.10	0.12	0.11	0.12	0.05
Mg ²⁺	0.19	0.22	0.69	0.37	0.17	0.11	0.05
Mn ²⁺	0.02		0.01	0.02		0.08	0.01
Na ⁺	0.37	0.01	0.02	0.02	0.04	0.08	0.05
K ⁺	0.18	0.22	0.08	0.09	0.12	0.24	0.41
	0.92	0.51	0.90	0.62	0.44	0.63	0.57
Layer charge	1.33	0.82	1.73	1.16	0.74	0.96	0.70
Interlayer	1.29	0.79	1.70	1.13	0.72	0.94	0.68

* Analyses by Cameca Camebax electron microprobe. Values reported as mean and s.d. of at least three spot analyses per sample.

† All analyses normalized to total of Loihi sample no. 1. Totals varied from 74.2 to 85.3%, due largely to variable water contents of the nontronite.

‡ Calculated on the basis of 20 oxygens and 4 hydroxyls per formula unit and assuming all Fe as Fe³⁺.

§ Calculated with Fe/Si ratio of CBD-treated Loihi no. 2 sample.

Persistent swarms of earthquakes, characteristic of those associated with Hawaiian volcanism, occur near the summit at depths of 0–10 km below the sea floor. This seismic activity is not associated with Kilauea or Mauna Loa activity, suggesting that Loihi submarine volcano marks the site of a separate, new locus of earthquake activity and volcanism along the Hawaiian Ridge³.

Previous bottom photographic surveys and dredging on the Loihi summit have shown that the ocean floor is covered by fresh-looking, coherent pillowed lava flows, pillow joint block talus and pockets of sand and gravel². Moore *et al.*² observed that all of the young, glass-encrusted pillow fragments recovered during dredging were covered with a thin, iron-rich, red-brown coating possibly of hydrothermal origin.

To determine the nature of hydrothermal activity on the summit of this hotspot volcano, we carried out a field programme and analysis of bathymetric data collected by the US Navy Ship *H. H. Hess* over the seamount, hydrographic data collected by the NOAA ships *Fairweather* and *Rainier*, and bottom photography taken with the Angus camera system aboard the University of Hawaii vessel *R/V Kana Keoki*. We then chemically analysed the hydrothermal sediments collected by transponder-navigated dredge hauls over the active summit rift areas.

Geological setting of Loihi

Volcanoes of the Hawaiian hotspot are located along the axes of a double trace⁴ (Fig. 1). Jackson *et al.*⁴ showed that volcanic activity has alternated regularly between the two traces while advancing south under the northwestward moving Pacific plate.

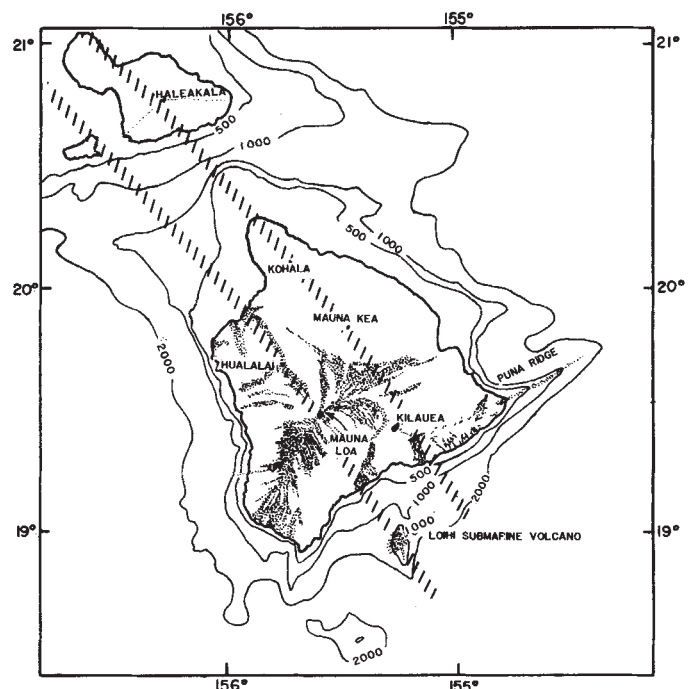


Fig. 1 'Hotspot' traces (hatchures) of the volcanoes of the island of Hawaii, showing the location of Loihi Seamount and the sites of recent lava flows (dotted patterns) along the rift zones of Mauna Loa and Kilauea volcanoes.

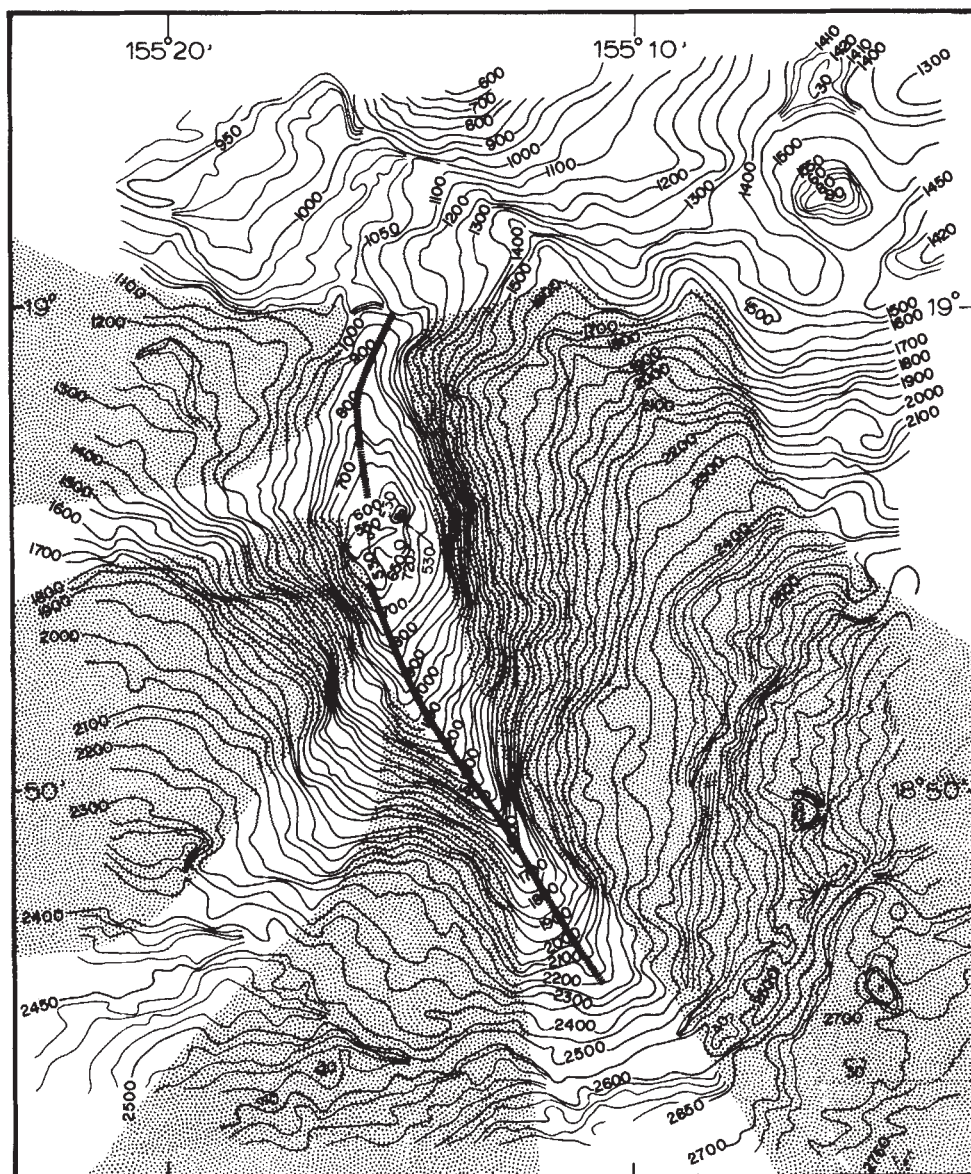


Fig. 2 Bathymetric map of Loihi, constructed from SASS narrow beam, multi-beam data. Dotted pattern shows flanks of the volcano that have probably been subjected to mass wasting through slumping and talus formation. Hachures show location of the two summit rift zones. Depth contours in fathoms (1 fathom = 1.83 m).

Loihi volcano appears to be connected with the Kahoolawe-Hualalai-Mauna Loa trace and not with the southern extension of the Haleakala-Kohala-Mauna Kea-Kilauea trace (Fig. 1).

A high-resolution multi-beam (SASS) bathymetric survey of Loihi was conducted during January 1981 (Fig. 2). The volcano has formed along a south-east-striking rift, ~30 km long. An elongate volcanic edifice extending from a water depth of 4,026 m (2,200 fathoms) to 980 m was formed as a result of persistent submarine volcanism. The summit rift area of Loihi (Fig. 2) consists of three structural elements—a north rift, a summit crater and a south rift.

The summit crater is offset to the east of the north-south rift system. Detailed examination of the multi-beam bathymetric data shown in Fig. 2 and results of the Angus bottom camera traverses show that lava has erupted recently from rifts along the southern edges of the summit crater. The upper edge of the rim is overlain by fresh pahoehoe lava, the steeper slopes of the crater by aa flows and the gentler lower slopes by pillow flows. The lowest slopes photographed, located at a water depth of 1,650 m, are entirely covered by fresh talus, suggesting that the dominant geological process is summit volcanism followed by a breakup of the young flows along the flanks of the volcano. The steep lower slopes of Loihi and the cirque-shaped indentations of its flanks (Fig. 2) probably have been formed as a result of mass downslope wasting of talus, a geological process well

documented for the submarine portions of the Hawaiian islands². A concurrent process of summit growth by volcanism and flank destruction by talus formation and downslope talus wasting has probably produced the elongated, steep-flanked dome of Loihi.

The summit of Loihi is marked by an elongate crater and rift system (Fig. 3). The crater is ~2,800 m wide and 3,700 m long and resembles the craters and calderas of sub-aerial Hawaiian volcanoes. Two prominent craters, 275 and 256 m deep, respectively (Fig. 3), within the summit crater, resemble the pit craters seen along the sub-aerial rifts of Kilauea. Bottom photographs show that the floor of the Loihi pit craters is covered by talus and sediments.

Extensive fresh lava flows, several hydrothermal fields and water temperature anomalies were observed with the Angus system over the southern rim of the summit crater, principally at the intersection of the rim with the south rift. An extensive hydrothermal field associated with recent lava flows (identified by the glassiness of the lavas) was mapped along the inner walls of the southern rim of the crater (Fig. 3). This particular hydrothermal field was characterized by a water temperature anomaly of >1 °C recorded at a water depth of 10 m above the ocean floor, by the presence of swarms of either bathypelagic organisms or clumps of benthic microorganisms in the water column (recorded by the camera) and by the presence of fields of

Table 2 Trace element chemistry of Loihi Seamount hydrothermal deposits

Sample	Al (%)	Fe (%)	Mn (p.p.m.)	Cu (p.p.m.)	Ni (p.p.m.)	Pb (p.p.m.)	Cd (p.p.m.)	As (p.p.m.)	Hg (p.p.b.)	Fe/Mn	Cu/Al ($\times 10^4$)	Ni/Al ($\times 10^4$)
Loihi no. 1 bulk	1.22	33.8	1,690	650	215	21	3.5	11	68	200	533	176
Loihi no. 2 <44 μ m CBD	1.81	22.8	<200	146	61	19	0.89	<2	38	>1,100	81	34
Loihi no. 2 <44 μ m CBD extract	0.28	10.5	430	72	27	2.6	0.28	3.3	11	244	257	96
EPR Fe deposit*	<0.5	30.8	16,500	85	320	—	—	—	—	19	>170	>640
Galapagos nontronite†	0.02	24.5	1,900	5.2	8.5	—	—	4.1	—	129	260	425
Galapagos nontronite‡	0.16	20.8	900	64	20	47	—	—	—	231	400	125
Famous clay-rich deposit§	0.13	25.1	28,950	63	40	—	—	—	—	8.7	485	308

Analyses by Perkin Elmer model 603 atomic absorption spectrophotometer. Cd and Pb by graphite furnace; As and Hg by hydride generator; all others by standard flame techniques. Instrumental precision was: Al $\pm 0.05\%$, Fe $\pm 0.1\%$, Mn ± 20 p.p.m., Cu ± 10 p.p.m., Ni ± 5 p.p.m., Pb ± 0.5 p.p.m., Cd ± 0.01 p.p.m., As ± 1 p.p.m., Hg ± 10 p.p.b.

* Average of samples a₁, a₂ and a₃ from dredge AMPH D2 (ref. 12).

† Average of samples N-1 and N-2 (ref. 19).

‡ Average of 424-2-2, 11–13 cm; 424A-2-2, 9–11 cm; and 424B-2-2, 50–52 cm (ref. 20).

§ Average of dark green and yellowish green samples of CYP74-26-15 and 16 (ref. 21).

yellow-coloured deposits apparently precipitated onto the talus slope (Fig. 4). The precipitation of the deposit probably occurred as a result of hydrothermal fluid seepage through the talus pile. The locations of the hydrothermal fields described and sampled are shown in Fig. 3.

Analyses of the hydrothermal deposits

The deposits dredged from the Loihi Seamount hydrothermal fields consist of yellow-brown, well crystallized goethite and smectite. (The observed colour range from red² to yellow is probably due to the changing oxidation state of the iron in these deposits.) The two samples are similar except perhaps for the relative amounts of goethite, which is suggested in their X-ray diffraction peak intensities (not shown) and Fe contents (Table 1). Figure 5 shows the X-ray diffraction patterns of the two size fractions of Loihi sample 2. A 15.5-Å dioctahedral smectite, which expands to 18 Å on glycolation, is the predominant phase in both size fractions of this sample. The smectite is a nontronite, similar in composition to the Fe-rich montmorillonite-nontronite recovered from mid-ocean spreading centres such as the Red Sea Rift⁵, the East Pacific Rise⁶ and the Galapagos hydrothermal mounds field (Table 1). The composition and morphology of the Loihi deposits is distinct from that of the surrounding deep-sea sediments on the Hawaiian Arch, which are largely composed of siliceous biogenic debris and volcanic ash⁷.

Table 1 presents the structural formulas of the Loihi nontronite and of two selected nontronites from the Galapagos hydrothermal mounds field. The Loihi nontronite appears to have a structural formula between those of the two Galapagos nontronites, resembling that of 424A-1-1 in the tetrahedral and interlayers and that of 424B-2-4 in the octahedral layer. In general the Loihi nontronite contains more structural Fe than the most Fe-enriched Galapagos sample analysed by us to date (424B-2-4), whereas the Galapagos nontronites contain more interlayer K and possibly Mn (Table 1).

The large amount of tetrahedral Fe found in the <44- μ m fraction of Loihi sample 2 is probably from inclusion of very fine-grained iron oxide contaminants (goethite), as this tetrahedral Fe disappears along with the goethite X-ray peaks when the sample is treated with a sodium citrate-bicarbonate-dithionite buffer solution (CBD)⁸ (Table 1, Fig. 5). Using the Fe/Si ratio of the CBD-treated split of sample 2, we have calculated the abundance of excess iron oxide, or goethite, present in Loihi sample 1 and the <44- μ m fraction of Loihi

sample 2. These calculations yield 61.5% goethite (oven dried at 110 °C) for Loihi sample 1, and 16.4% goethite for the <44- μ m fraction of Loihi sample 2. The resultant structural formulae for the nontronite are presented in Table 1. The high interlayer Mg content of Loihi sample 2, <44 μ m, is due to Mg saturation during sample preparation. An *in situ* interlayer composition is probably represented by those of Loihi sample 1 and the >44- μ m fraction of Loihi sample 2, which received no chemical treatment before analysis.

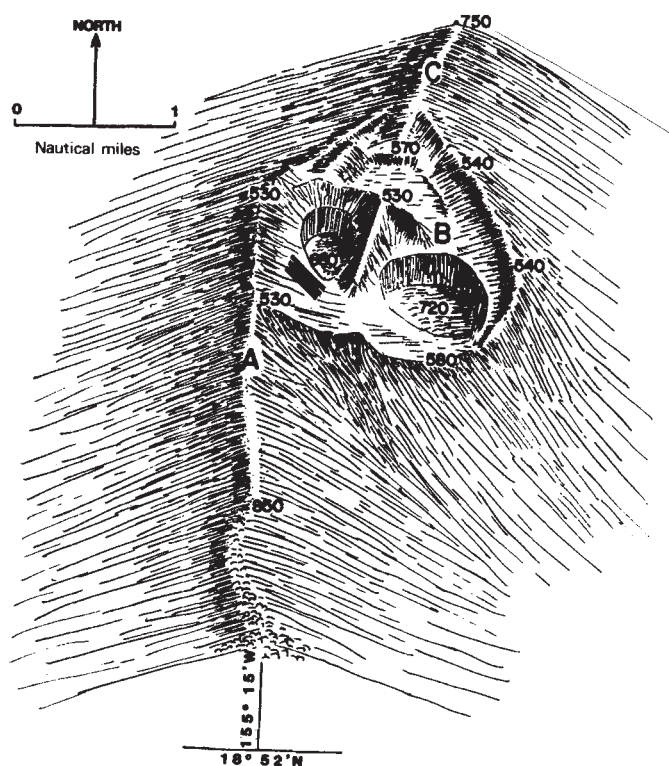


Fig. 3 Perspective physiographic drawing of the summit of Loihi submarine volcano. Spot depths in fathoms. Principal features are: A, south-east rift; B, summit crater with axial ridge and two pit craters; and C, north rift. Dashed lines indicate locations of dredged samples of fresh flow basalt and nontronite from the summit hydrothermal vents.

The Al values determined by atomic absorption spectrophotometry (AAS) are consistently higher than those determined by the electron microprobe (Tables 1 and 2). This discrepancy and that for Fe in Loihi sample 1 are probably because the electron microprobe analyses are highly selective, although different values may also be due in part to variable water contents of the nontronite. Nevertheless, the AAS results confirm the high Fe/Mn ratios of the Loihi deposit (Tables 1 and 2). The Loihi deposit is enriched in Cu relative to hydrothermal deposits from the East Pacific Rise, Galapagos Rift and Mid-Atlantic Ridge, but values are similar for Ni, Pb and As. Aluminium is more abundant in both the nontronite and Fe-oxide fractions of the Loihi deposit than in most of the other hydrothermal deposits (Table 2). The Cd value of 3.5 p.p.m. for Loihi sample 1 is higher than those for Hawaiian basalts by an order of magnitude [0.13–0.49 p.p.m., eight samples analysed (G.M.McM. and J.C.W., unpublished data)], whereas the Hg values are within the range of Hawaiian basalt levels [17–67 p.p.b. (parts per 10⁹), eight samples analysed].

Oxygen isotope analysis of the CBD-treated, <44- μ m fraction of Loihi sample 2 yields a value of $+24.4 \pm 0.2\%$ for the nontronite. Assuming a $\delta^{18}\text{O}$ of 0.0 for seawater, the geothermometric equation for the smectite–water system⁹ gives a formation temperature of $31 \pm 1^\circ\text{C}$ for the nontronite. This isotopic value and the formation temperature are in good agreement with those found for Fe-rich montmorillonites from the East Pacific Rise and Bauer Basin⁶ and with those found for nontronites from the Galapagos hydrothermal mounds field (G.M.McM. and H.-W.Y., unpublished data, and T. J. Barrett, personal communication). Because temperature anomalies of $>1^\circ\text{C}$ were measured by deep-towed vehicles 10–20 m above the Galapagos Rift and East Pacific Rise where vent temperatures of $\geq 30^\circ\text{C}$ were later measured from submersibles¹⁰, the similar temperature anomalies over the Loihi hydrothermal fields measured by the Angus system therefore suggest the presence of vent temperatures of $\geq 30^\circ\text{C}$ on the ocean floor. This range of inferred temperatures is also in general agreement with the $\delta^{18}\text{O}$ temperature of formation for the nontronite.

Discussion

Submarine hydrothermal spring deposits that are enriched in iron, manganese and trace transition metals have been described from submarine volcanoes in the South Pacific^{11,12}

and from hot springs discharge into seawater from nearby subaerial volcanoes¹³. The mineral assemblage—goethite and Fe-montmorillonite–nontronite—that characterizes the Loihi seamount deposits has been recognized as an important component of the Red Sea hydrothermal brine deposits^{3,14} and of the extensive hydrothermal deposits in the Bauer Basin^{15–17}.

On the crest of the East Pacific Rise spreading centre, Fe-montmorillonite is generally found with 40–60% X-ray amorphous Fe–Mn oxides^{6,18}. The Galapagos hydrothermal mounds are primarily composed of nontronite with surficial crusts of well-crystallized Mn oxides^{19,20}. Hydrothermal deposits found near fossil vents in the *Famous* area of the Mid-Atlantic Ridge are composed of hydro-mica, smectite and an X-ray amorphous Fe–Si compound; most of the deposits farther from the vents are comprised of Fe–Mn concretions²¹.

McMurtry and Yeh⁶ calculated oxygen isotopic formation temperatures of 30–50 $^\circ\text{C}$ for the Fe-montmorillonite from the East Pacific Rise and Bauer Basin, confirming the low-temperature hydrothermal formation of this mineral. The mode of hydrothermal formation may be either: (1) precipitation of monomeric SiO_2 and dissolved Fe (and other cations) from cooling vent fluids in mildly reducing conditions, as suggested for the Red Sea brine and Galapagos mounds nontronites^{14,19}; or (2) precipitation during cooling and oxidation of high-temperature, sulphide-dominated assemblages (retrograde metamorphism progressing towards low-temperature diagenesis)⁶. The X-ray amorphous Fe-oxide or goethite is generally considered a simultaneous or post-smectite oxidation product.

We interpret the Loihi hydrothermal deposits as (1) precipitates that formed on discharge to the sea floor, or (2) low-temperature metamorphic or diagenetic products of high-temperature sulphide chimneys such as those discovered on the East Pacific Rise at 21 $^\circ\text{N}$ ^{10,22,23} and on the Galapagos Rift²⁴. A possible constraint on the correct mode of formation is the near absence of manganese in the Loihi deposits (Tables 1 and 2).

The type of metal-rich deposits formed at submarine hydrothermal centres—either iron–copper–zinc–sulphide rich/manganese depleted; iron–manganese rich; or manganese rich, iron–copper–zinc depleted—depends largely on the progressive mixing of the primary, high-temperature ($\sim 400^\circ\text{C}$), acid, reducing solution with ambient seawater at depth in the basalt pile. The Fe/Mn ratio may further depend on the iron/sulphide ratio in the end member hydrothermal solution and/or the glass/phenocryst ratio in the altered basalt²⁵. The extremely high Fe/Mn ratio of the Loihi hydrothermal deposits and, to some extent, the Cu and Cd enrichments suggest that the deposits were formed either from the alteration of or in close association with high-temperature sulphide assemblages, either at depth in the volcano or near the exit vents. Indeed, fields of small chimneys (≤ 1 m high) consisting of grey material and protruding from mound-like structures were photographed and mapped (but eluded shipboard sampling) in the vicinity of the nontronite fields from which the two samples were taken²⁶. On the other hand, the lack of manganese may have resulted from its greater solubility, causing fractionation of iron from manganese and variable Fe/Mn ratios, as suggested for dredged seamount deposits from the East Pacific Rise¹². In contrast to the East Pacific Rise dredges, however, the Loihi dredges contained no evidence of manganese-enriched crusts expected to occur as distal deposits.

The extremely high Fe/Mn ratio of the deposit and the lack of manganese-encrusted lavas in the dredged samples suggest that either (1) the Loihi hydrothermal system is a high-temperature sulphide producer; (2) the iron/sulphide ratio in the hydrothermal fluid and/or the glass/phenocryst ratio in the basalt is unusually high; or (3) manganese dispersal is very efficient here, possibly because of the increased effects of bottom currents on a seamount as contrasted to a mid-ocean rift valley. The geochemical data suggest that the Loihi hydro-

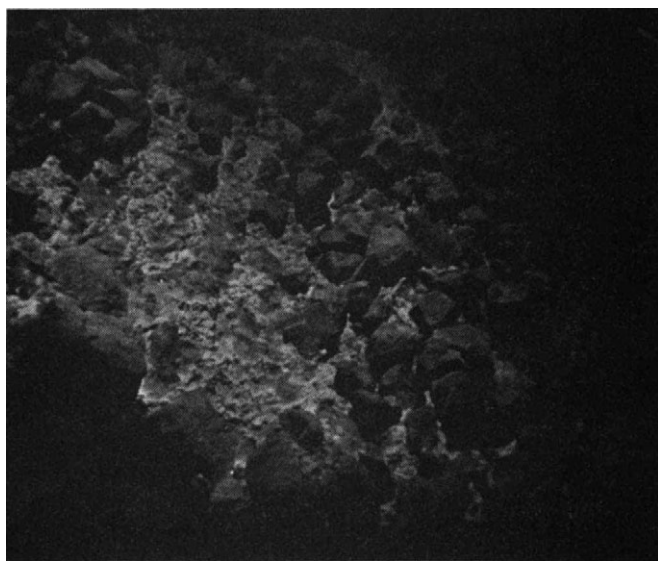


Fig. 4 Nontronite deposited onto a talus slope at the summit of the active submarine volcano Loihi, the southernmost expression of the Hawaiian 'hotspot'. This photograph (representing a width of 4 m) was taken with the Angus system over a currently active hydrothermal field at a water depth of 1,080 m.

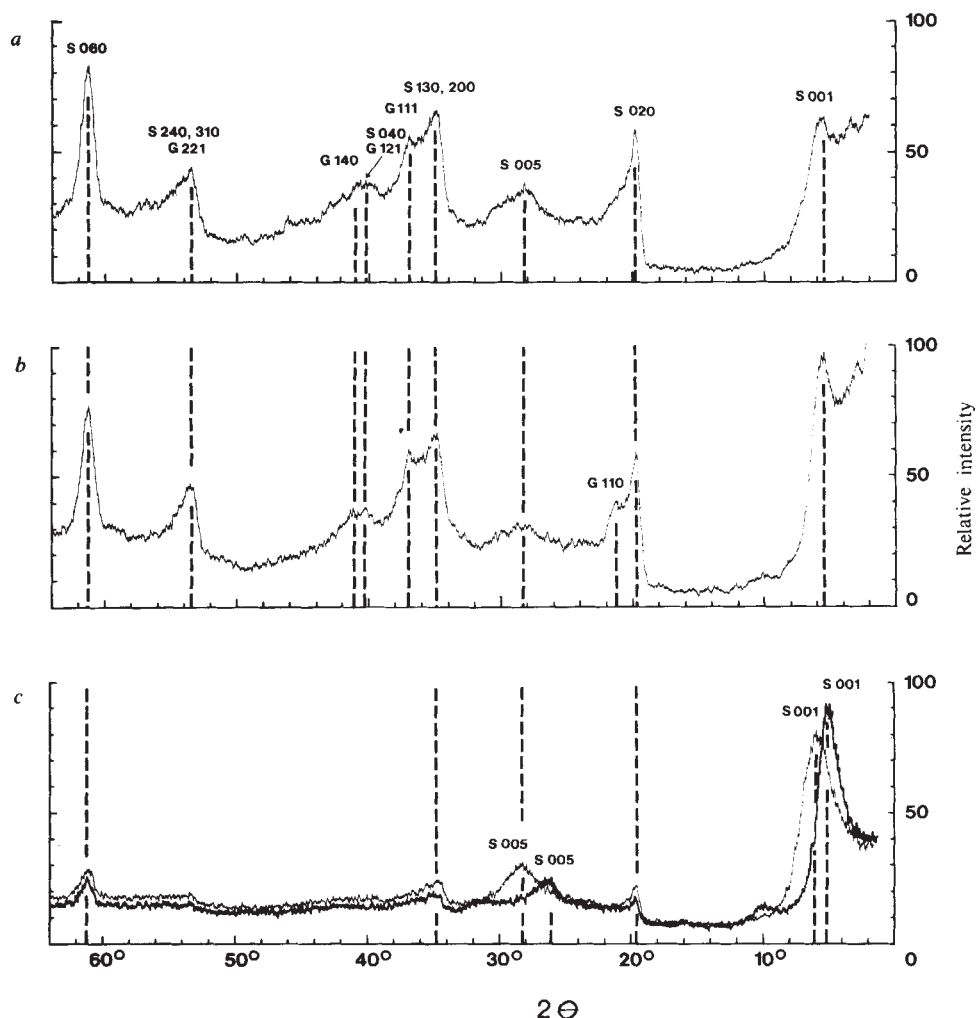


Fig. 5 *a*, X-ray diffraction pattern of Loihi sample 2, $>44\ \mu\text{m}$. Oriented mount, scan speed at $2^\circ\ (2\theta\ \text{min}^{-1})$. S, smectite; G, goethite. *b*, X-ray diffraction pattern of Loihi sample 2, $<44\ \mu\text{m}$. Oriented mount, scan speed at $2^\circ\ (2\theta\ \text{min}^{-1})$. S, smectite; G, goethite. *c*, X-ray diffraction pattern of Loihi sample 2, $<44\ \mu\text{m}$. CBD treated. Dark trace: after 24-h exposure to ethylene glycol. Oriented mount, scan speed at $2^\circ\ (2\theta\ \text{min}^{-1})$.

thermal deposits could contain high-temperature, polymetallic sulphides at depth, in the volcanic pile.

Although bottom photography showed a striking morphological resemblance of the Loihi hydrothermal fields to those of the Galapagos Rift²⁷, no exotic benthic fauna were found to be associated with the Loihi vents. Absence of such fauna suggests that submarine hydrothermal activity along a hotspot island chain such as the Hawaiian Islands is too infrequent to support a specialized, evolving, benthic community. Also, distances between mid-plate hydrothermal vents such as Loihi may be too great for larvae to migrate from their places of specialization along the crest of the East Pacific Rise, even though environmental conditions may be just as favourable for growth on Loihi as they are along the East Pacific Rise.

These results indicate that Fe-oxide and nontronite precipitation are related not only to the process of mid-ocean ridge volcanism but also to processes of active volcanism associated with submarine mid-ocean hotspot volcanoes. This relationship may be taken one step further to suggest that the Pacific mid-plate seamounts and islands could con-

tain hydrothermal sulphide deposits at depth, in the volcanic pile.

We thank the captains and crew of the NOAA vessels *Rainier* and *Fairweather* for detailed hydrographic surveys, and the US Navy and Dr William Hart of the Naval Oceanographic Office for the multi-beam SASS bathymetric survey over Loihi. Bottom photography was by the Angus bottom photography system operated during the October 1980 cruise to Loihi aboard the R/V *Kana Keoki*. The Angus team of the Woods Hole Oceanographic Institute was led by Earl Young. We thank Frisbee Campbell and Robert Ballard for encouragement. G.M.M. thanks M. O. Garcia and R. C. Jones for assistance with the electron microprobe and X-ray diffractometer. We thank Rita Pujale and Barbara Jones for editorial assistance. This research was supported by the National Oceanic and Atmospheric Administration, National Ocean Survey, and UH Sea Grant College Program under institutional grant NA81AA-D-00070, project ME/R-4. Hawaii Institute of Geophysics contribution 1210 and Sea Grant contribution UNIH-sea grant-JC-82-11.

Received 8 January; accepted 27 April 1982.

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Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products

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Alternative processing of RNA transcripts from the calcitonin gene results in the production of distinct mRNAs encoding the hormone calcitonin or a predicted product referred to as calcitonin gene-related peptide (CGRP). The calcitonin mRNA predominates in the thyroid while the CGRP-specific mRNA appears to predominate in the hypothalamus. These observations lead us to propose a model in which developmental regulation of RNA processing is used to increase the diversity of neuroendocrine gene expression.

A LARGE array of hormonal signals provides the specificity of intracellular communication within and between organs. The existence of gene families¹ and the presence of multiple hormones within a single primary translation product²⁻⁴ are two means by which a diversity of peptide hormones can be generated. The discontinuity of genetic regions encoding mature RNAs and the complexity of RNA processing pathways suggest that alternative splicing events could be an additional mechanism for increasing the flexibility of gene expression in the neuroendocrine system. The potential versatility provided by alternative RNA processing events has been used effectively by several eukaryotic viruses to generate multiple protein products from a single transcription unit^{5,6}. We show here that multiple mRNAs are generated from the calcitonin gene as a consequence of alternative RNA processing events. These events ultimately produce different protein products and seem to occur in a tissue-specific fashion. Such a mechanism could serve to increase the diversity of proteins encoded by endocrine genes.

The production of multiple calcitonin-related mRNAs was first noted during the spontaneous and permanent switching of serially transplanted rat medullary thyroid carcinoma (MTC) lines from states of 'high' to 'low' calcitonin production⁷. This conversion is associated with the appearance of new calcitonin cDNA-reactive mRNAs, referred to as calcitonin-gene-related product (CGRP) mRNAs, 50–250 nucleotides larger than calcitonin mRNA. These RNAs can function as mRNAs as they are present on polyribosomes and can be translated *in vitro* after being isolated by hybridization to a specific immobilized cDNA clone. A new 16,000 molecular weight protein is the major cell-free translation product directed by the CGRP mRNAs⁸; this protein does not contain immunoreactive calcitonin.

To study the switching events occurring in the tumours, cDNA clones to calcitonin (pCal) and calcitonin gene-related (pCGRP₁, pCGRP₂) mRNAs have been generated and a calcitonin genomic fragment referred to as λ -Cal₁ has been isolated. In this article we examine the structural basis for differences between mature calcitonin mRNA (Cal mRNA) and one of the variant mRNAs (CGRP mRNA) by examination of both cDNA and genomic clones.

Cal and CGRP mRNA sequences are present in the same gene

To determine the relationship of Cal and CGRP sequences within the calcitonin gene, fragments or clones bearing calcitonin-specific or CGRP-specific sequences were used as hybridization probes to locate and orientate exons within the genomic clone, λ -Cal₁. These regions of homology are closely linked as both probes react with a common 8.2-kilobase (kb) *Eco*RI fragment and a common 3.0-kb *Pst*I fragment (Fig. 1a, b). Fragments specific to 5' and 3' regions of calcitonin mRNA were generated as hybridization probes to determine the transcriptional orientation of the gene. These were prepared by cleaving pCal at the single *Bgl*II site, located 11 nucleotides 5' to the start of the calcitonin coding sequence⁹. A cDNA clone pCGRP₁ was used as a specific probe for CGRP sequences because it contains a region unique to CGRP mRNAs and does not hybridize Cal mRNA. The pCal- and pCGRP₁-reactive *Eco*RI and *Pst*I fragments of λ -Cal₁ correspond precisely to those identified in digests of genomic DNA isolated from rat liver or MTC tumours. Figure 1c, d shows the results of this analysis. Comparison of this hybridization with that in a and b indicates that CGRP sequences map to the 3' portion of the gene. Further restriction mapping establishes that the 8.2-kb *Bgl*II fragment which hybridizes only with pCGRP₁ (Fig. 1a, lane 2) represents the 3' end of the λ -Cal₁ clonal insert. These results support the notion that the 3' ends of Cal and CGRP mRNAs are encoded by the same gene.

Comparison of pCGRP₂ and pCal cDNA coding domains

Previously described RNA hybridization analysis⁸ and the genomic mapping observations presented above suggest that Cal and CGRP mRNAs contain common or homologous 5' sequences and nonhomologous 3' sequences. This prediction was tested by restriction analysis and sequencing of CGRP cDNA clones. One clone, designated as pCGRP₂, containing an insert of >600 nucleotides, was sequenced to identify the

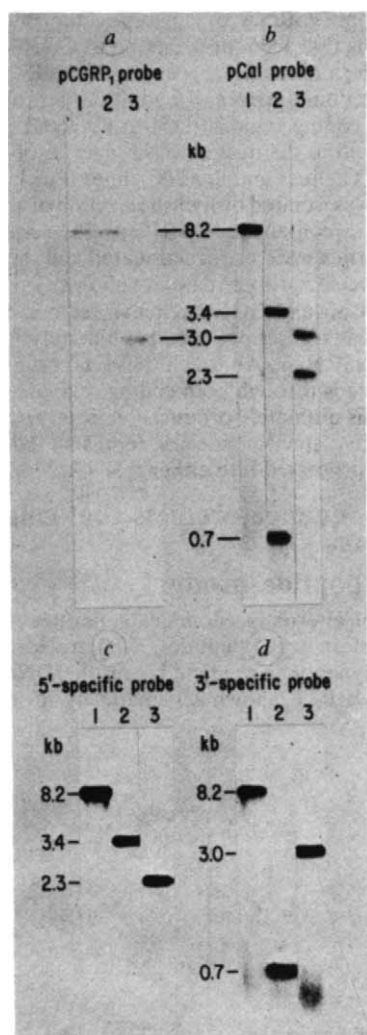


Fig. 1 Localization of pCGRP sequences in the calcitonin genomic clone λ -Cal₁. The rat calcitonin genomic clone (λ -Cal₁) was digested to completion with the restriction enzymes *Eco*RI, *Bgl*II or *Pst*I and electrophoresed on an 0.8% agarose gel. The restriction fragments were transferred to a nitrocellulose filter²⁷. Filters were hybridized to $\sim 5 \times 10^6$ c.p.m. ml⁻¹ of ³²P-labelled nick-translated probes, washed and autoradiographed as previously described⁸. The clone pCGRP₁ used in this analysis contains a region unique to CGRP mRNA and does not hybridize to Cal mRNA. *a*, Hybridization of pCGRP₁ to λ -Cal₁ digested with: *Eco*RI (lane 1), *Bgl*II (lane 2), *Pst*I (lane 3). *b*, Hybridization of pCal to a duplicate blot of *a*. *c*, Hybridization of the 5' fragment of the *Bgl*II-digested pCal insert to λ -Cal₁ DNA digested with: *Eco*RI (lane 1), *Bgl*II (lane 2), *Pst*I (lane 3). *d*, Hybridization of the 3' fragment of *Bgl*II-digested pCal insert to the identical blot shown in *c*. Sizes are based on the migration of *Hind*III-digested λ DNA fragments.

point of divergence between Cal and CGRP mRNAs. The sequence of this clone reveals the entire coding region of CGRP mRNA with an open reading frame of 384 nucleotides capable of coding for a primary translation product of 128 amino acids.

The sequences of pCGRP₂ and pCal are compared in Fig. 2a. The 5' sequences of pCal and pCGRP₂ are identical from the first nucleotide in the pCGRP₂ insert to nucleotide 227 in the coding region of both cDNA clones. As previously reported, the structure of pCal predicts only one open reading frame which encodes a precursor protein whose characteristics correspond to those determined for precalcitonin and its component polypeptides *in vivo* and *in vitro*⁹⁻¹¹. Thus, the identity of the 5'-proximal mRNA sequences including the AUG for both mRNAs suggests that the first 76 amino acids of their translation products must also be identical. The nucleic acid sequence diverges 228 nucleotides into the coding sequence, following an AG, and precisely identifies the junction point that distin-

guishes these two mRNAs. Beyond nucleotide 227, the two mRNAs share no homology and encode completely different amino acid sequences. The junction of shared and divergent sequence in these two mRNAs is detailed in Fig. 2c. These data are most compatible with the interpretation that a single 5' sequence represents a discrete domain which can be selectively spliced onto Cal- or CGRP-specific exons. In such an instance, the mRNA junction point must correspond to an intervening sequence in which a unique donor site can splice onto one of two alternative acceptor sites. We examined this prediction by a detailed structural analysis of the calcitonin gene.

The point of divergence between Cal and CGRP mRNAs corresponds to a genomic splice junction

Detailed restriction endonuclease mapping using the cDNA clones as probes and partial DNA sequence analysis of the calcitonin genomic clone (λ -Cal₁) confirms the prediction that exons with sequences precisely corresponding to the divergent 3'-coding regions of pCal and pCGRP₂ are present in one genomic transcription unit. As noted in Fig. 2b, all exons identified in the genomic clone have sequences identical to those determined for the two cDNA clones. The 5' boundaries of the Cal- and CGRP-specific genomic coding sequences establish that splice junctions occur precisely at the point of the divergence between Cal and CGRP₂ mRNAs. Figure 2c (A) and (B) details the sequences at the junctions for the pCal and pCGRP₂ sequences. The observation that an AG precedes both exons in the genomic clone is in accordance with the GT...AG rule for splice junctions¹² and is consistent with the prediction that each exon can be alternatively spliced into the mature mRNAs. The AG which appears adjacent to the splice site in the two cDNA clones is presumably contributed by the 3' end of the preceding exon.

Nuclear RNA transcripts contain both Cal and CGRP sequences

The structure of Cal and CGRP mRNAs and of the calcitonin genomic clone demonstrate that the same region of DNA can be used to generate multiple mature mRNAs. However, there are several possible mechanisms by which each mature mRNA could be produced. Because the sequences unique to CGRP mRNA are located 3' to Cal mRNA sequences in the gene, it is possible that the calcitonin primary transcript could terminate at a polyadenylation site 5' with respect to CGRP sequences, while transcription to the next polyadenylation site could give rise to the CGRP primary transcript. This model predicts two different primary transcripts and would parallel the molecular events demonstrated in the case of late adenovirus mRNA production; these events have also been proposed for the alternative generation of membrane and secretory forms of immunoglobulin μ heavy chains^{13,14} and the simultaneous synthesis of μ and δ heavy chains¹⁵.

This possibility was investigated by analysing putative nuclear RNA precursors for the presence of both Cal and CGRP mRNA-specific sequences. We have previously identified reactive pCal RNA precursors 6.6, 5.2, 4.2 and 3.8 kb in tumour lines producing CGRP calcitonin mRNA (MTC_L lines)⁷. The comparably sized precursors observed in lines producing predominantly calcitonin mRNA (MTC_H lines) could reflect the production of small amounts of CGRP mRNAs⁸, the existence of common precursors for Cal and CGRP mRNAs, or the presence of CGRP RNA precursors not processed to mature CGRP mRNAs in this tissue. RNA from normal thyroid glands was also analysed. Virtually all the pCal-reactive RNA detected in the thyroid gland is the 1,050-nucleotide calcitonin mRNA. The presence of CGRP-specific sequences within the large

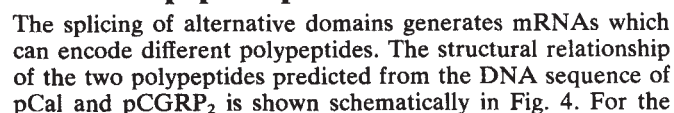
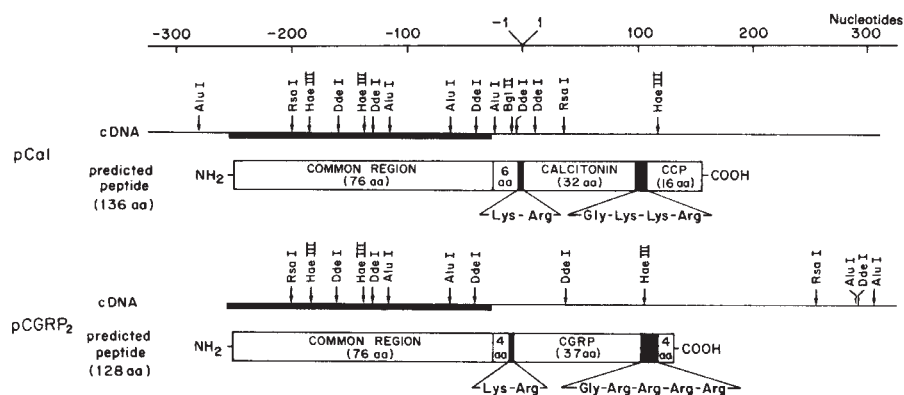


Fig. 3 *a*, Analysis of calcitonin mRNA precursors for the presence of CGRP regions. Total poly(A)-rich RNAs from an MTC_L line and from normal thyroids were denatured and electrophoresed on 1.5% agarose formaldehyde gel as described previously³³. RNA was transferred to nitrocellulose²⁷, washed in prehybridization buffer and hybridized to pCGRP, nick-translated to a specific activity of 8×10^8 c.p.m. per μ g using [α -³²P]dCTP as the labelled nucleotide. The pCGRP probe is specific for CGRP sequences. Lane L, 5 μ g MTC_L tumour mRNA; lane T, 5 μ g thyroid mRNA. *b*, Analysis of hypothalamic and thyroid mRNA species hybridizing the calcitonin cDNA clone pCal. Poly(A)-rich mRNA from normal thyroid and gradient-enriched 8–15S RNA from rat hypothalamus were subjected to formaldehyde gel and Northern blotting procedures as in *a* except that transfer was to diazotized paper³⁴. Immobilized RNA was hybridized with pCal nick-translated to a specific activity of 5×10^8 c.p.m. per μ g (ref. 33). Lane 1, 5 μ g poly(A)-rich thyroid mRNA; lane 2, 8 μ g gradient-enriched poly(A)-rich hypothalamic mRNA. RNA sizes are based on migration of 18S and 28S RNAs.

Fig. 4 Schematic representation of pCal and pCGRP₂ DNA inserts and the structure of the precursors they encode. Restriction sites used for DNA sequence analysis are those indicated and regions of identical nucleotide sequence are underlined in black. Nucleotide residues numbered on the scale above begin with the first residue in the coding region for calcitonin. The structure of the predicted protein precursors are represented by the bar below. Potential proteolytic processing sites and the resulting cleavage products are also noted.



calcitonin precursor, the points of excision can be accurately determined from knowledge of the mature calcitonin peptide, as we have previously discussed in detail^{9,10}. The excision occurs at paired or multiple basic residues and this processing information is encoded in the calcitonin exon. The DNA sequence of pCGRP₂ predicts a 128 amino acid, 16,000 molecular weight protein which has the same 76 amino acid N-terminal sequence as the calcitonin precursor. The 52 C-terminal residues of the predicted protein contain similar processing signals which would allow excision of a 37 amino acid peptide. The Lys-Arg dipeptide, encoded by nucleotides 241–246 (see Fig. 2a), is proposed to represent the N-terminal cleavage site for excision of this novel product. The presence of glycine followed by three basic amino acid residues is thought to serve as a signal for C-terminal amidation and cleavage in three amidated peptides for which the structure of the precursor is known^{4,9,16}. The sequence Gly-Arg-Arg-Arg-Arg in the product encoded by CGRP mRNA may signal the generation of a peptide terminating in a phenylalanine-amide. We refer to this 37 amino acid excised peptide as calcitonin gene-related peptide (CGRP). We note that in parallel with calcitonin, the CGRP exon encodes all the processing signals necessary to generate CGRP.

Expression of Cal and CGRP mRNAs is tissue specific

The demonstration that the 'switching' of MTC tumours from production of Cal to CGRP mRNA is the consequence of alternative RNA splicing events prompted the speculation that such events might occur physiologically during differentiation. This possibility was examined by size-fractionation of RNA from several tissues and challenge with Cal- and CGRP-specific probes. The results of one such study are shown in Fig. 3b. The hypothalamus contains poly(A)-rich RNAs which hybridize specifically with the Cal-specific probe and which are the same size as the CGRP RNA species present in the MTC_L tumours (Fig. 3b, panel 2). These RNA species hybridize to the CGRP-specific probe. A small amount of RNA corresponding to calcitonin mRNA is also present in these cells while virtually all the reactive RNA in the thyroid is calcitonin mRNA (compare Fig. 3a, panel T, with Fig. 3b, lane 1). These data suggest that the alternative RNA splicing events might occur physiologically in the expression of the calcitonin gene. It is tempting to speculate that CGRP, the predicted polypeptide product of CGRP₂ mRNA, is a hypothalamic neuropeptide that may exert hormonal effects of its own.

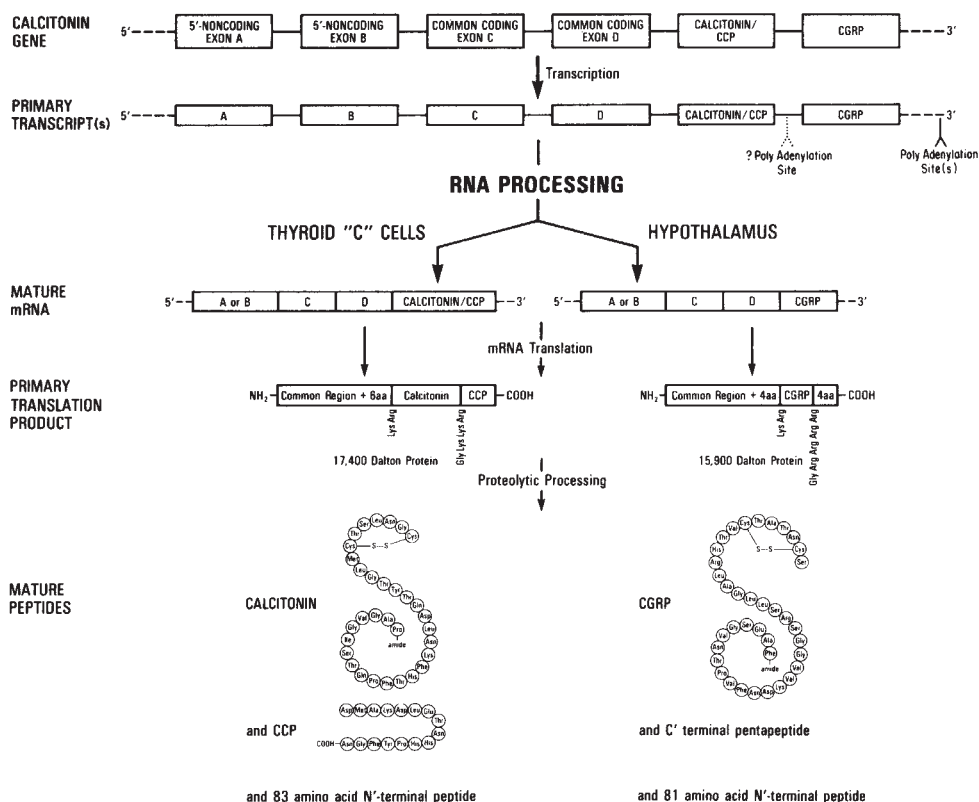


Fig. 5 A model to describe the tissue-specific expression of the calcitonin gene during 'peptide switching' events. The 5' and 3' termini are indicated as dotted lines because their structural organization remains unknown. The structural organization of coding blocks A and B, and the presence of A within CGRP mRNAs are not unequivocally established. Possible sites of polyadenylation of the primary transcript(s) are indicated. The forked arrow indicates tissues in which pCal reactive RNAs predominate (thyroid) or pCGRP reactive RNAs predominate (hypothalamus). The predicted proteolytic products of the primary translation product directed by Cal mRNA are established, those of CGRP mRNA, predicted.

Concluding remarks

The discovery of 'split genes' and definition of hnRNA structure^{5,6,17-21} raised the possibility of important regulatory events at the level of processing of initial RNA transcripts. As presented here, such regulation of RNA processing events may be necessary for the expression of certain neuroendocrine genes and may increase the diversity of the resultant peptide products. Genomic mapping data are consistent with the existence of a single calcitonin gene⁸; if a second gene exists it would have to be extremely similar in size and organization. Sequence analysis establishes that Cal and CGRP domains are linked in a single genomic clone whose exons correspond precisely in sequence to the cloned mRNAs. Based on the structure of this gene and its RNA products, we propose a model in which genomic regions can represent discrete hormone encoding domains whose ultimate expression is dependent on differential RNA processing events (Fig. 5). Thus, these domains may be included or excluded during the maturation process to generate multiple mRNAs encoding alternative hormones or neuropeptides. We refer to the consequences of these RNA processing events as 'peptide switching' because the splicing of alternative exons results in the expression of different peptides.

Several explanations could account for the alternative expression of Cal and CGRP mRNAs. First, there could be two overlapping transcription units with different cap and poly(A) sites. Second, there could be a single cap site with two or more poly(A) sites. Multiple RNAs would arise from a choice of poly(A) site either during transcription or due to post-transcriptional cleavage and re-polyadenylation. Third, there could be a small rearrangement or modification of gene structure affecting a splice site or polyadenylation signal. Finally, there could be true regulation of splicing choice for a single primary transcript.

The last possibility would parallel events observed in SV40 and adenovirus gene expression, where several mRNAs can be generated with common 5' and 3' termini and yet encode different proteins^{5,6}. This process requires the differential use of multiple and optional 5' and 3' splicing sites. It has been suggested that the L-1 transcription unit of adenovirus displays splice-choice control²². Should such an analogy apply to expression of calcitonin it remains to be defined how such a process is regulated. Splicing regulation could arise from changes in enzyme specificity, small nuclear RNAs and/or other factors directly affecting the splicing process and could account for the accumulation of the larger nuclear RNA transcripts of the calcitonin gene during switching to production of CGRP mRNAs⁷.

Certain observations regarding the expression of the calcitonin gene must be accounted for in any model of alternative processing. Both the calcitonin genomic clone and the larger

nuclear RNA transcripts contain sequences specific to both the mature calcitonin and CGRP mRNAs. If the observed nuclear transcripts are in fact precursors of both mRNAs, then the expression of either Cal or CGRP mRNAs requires splicing out of an internal exon. This would be in contrast to the RNA processing events proposed in the case of immunoglobulin heavy chain gene expression, where a polyadenylation site intercedes between the exons involved in switching¹³⁻¹⁵. On the other hand, if a polyadenylation site is present between Cal and CGRP exons, the precursors of the two mRNAs will be distinct. In this case, the nuclear CGRP-reactive RNAs in the thyroid would represent species not destined to become either RNA or would require a cleavage and re-polyadenylation to be processed into calcitonin mRNA.

Although it has been proposed that, in the case of liver and salivary gland α -amylase and dihydrofolate reductase gene expression²³, differential 5' transcription initiation and/or alternative polyadenylation sites^{24,25} result in mRNA structural polymorphism, this does not affect the structure of the protein-encoding regions of the mature mRNAs. In fact, 5' heterogeneity also seems to occur in calcitonin mRNAs, where one splice junction precedes by nine nucleotides the initiator methionine codon (Fig. 2a). The location of this intervening sequence corresponds precisely with the 3' boundary of a region of 5' noncoding sequence in pCal that diverges from a sequence reported for another calcitonin clone²⁶. The use of 'optional' 5' exons, in addition to the coding exon switch discussed here, further increases the mRNA polymorphism associated with calcitonin gene expression, but does not alter the coding region of the resultant mRNAs.

The apparent tissue specificity of the observed splicing events implies developmental regulation of these processes. Calcitonin gene expression therefore represents a model system for exploring mechanisms responsible for both transcriptional and post-transcriptional regulation of specific gene expression during development. Whatever the responsible molecular mechanisms, the unexpected consequence of these RNA processing events is that the same gene which generates the hormone calcitonin in thyroid C-cells is apparently responsible for the expression of a new polypeptide product in the hypothalamus.

We thank Drs James E. Darnell, Francis Crick, Geoffrey Wahl, Jean-Jacques Mermod and Geoffrey Murdoch for advice and helpful discussions regarding the manuscript, Arnold Loera and Rodrigo Franco for providing experimental data, our collaborators Drs Bernard A. Roos and Roger Birnbaum for their continued support in these studies, Drs Tom Sargent and James Bonner for providing the rat liver DNA library, Maureen Brennan and Margaret Richards for preparation of the manuscript. This work was supported by grants from the American Cancer Society and the NIH.

Received 9 February; accepted 4 May 1982.

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Cloning and sequence analysis of cDNA for porcine β -neo-endorphin/dynorphin precursor

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The primary structure of a precursor protein that contains β -neo-endorphin, dynorphin and a third leucine-enkephalin sequence with a carboxyl extension has been deduced from the nucleotide sequence of cloned DNA complementary to the porcine hypothalamic mRNA encoding it. The three peptides are each bounded by Lys-Arg. This precursor protein, like adrenal preproenkephalin and the corticotropin/ β -lipotropin precursor, comprises multiple repetitive units and a cysteine-containing amino-terminal sequence preceded by a signal peptide.

SEVERAL endogenous peptides with opiate-like activity have recently been identified. In addition to the opioid pentapeptides methionine-enkephalin¹ (Met-enkephalin) and leucine-enkephalin¹ (Leu-enkephalin), there are larger opioid peptides containing a Met-enkephalin or Leu-enkephalin sequence as the core fragment. These include β -endorphin², which is Met-enkephalin with a carboxyl extension of 26 amino acids, and β -neo-endorphin³ and dynorphin^{4,5}, which are Leu-enkephalin with carboxyl extensions of 4 and 12 amino acids, respectively. We have previously elucidated the primary structure of the common precursor of corticotropin (ACTH) and β -lipotropin (β -LPH), from which β -endorphin is formed, by determining the nucleotide sequence of cloned DNA complementary to the mRNA purified from bovine pituitary intermediate lobes^{6,7}.

Recently, we have cloned cDNA for bovine adrenal preproenkephalin, using synthetic oligodeoxyribonucleotides for screening a cDNA library and for priming the reverse transcription of the mRNA, and have deduced the entire amino acid sequence of this precursor protein from the nucleotide sequence of the cloned cDNA⁸. A large portion of the structure of this protein has also been reported by Gubler *et al.*⁹, who used cDNA sequencing in conjunction with protein sequencing. Bovine adrenal preproenkephalin contains four copies of Met-enkephalin and one copy each of Leu-enkephalin, Met-enkephalin-Arg⁶-Phe⁷ (ref. 10) and Met-enkephalin-Arg⁶-Gly⁷-Leu⁸ (refs 8, 9, 11). This is also valid for human adrenal preproenkephalin, the primary structure of which has been predicted from the cDNA sequence¹² as well as from the corresponding genomic DNA sequence¹³. Because neither the ACTH/ β -LPH precursor (designated alternatively as prepro-opiomelanocortin) nor adrenal preproenkephalin contains the sequences of β -neo-endorphin and dynorphin, however, a precursor or precursors of these opioid peptides remained to be uncovered.

We have now cloned DNA sequences complementary to the porcine hypothalamic mRNA coding for both β -neo-endorphin and dynorphin. Nucleotide sequence analysis of the cloned cDNA has disclosed the primary structure of a precursor protein that contains β -neo-endorphin, dynorphin and a third Leu-enkephalin sequence with a carboxyl extension; this precursor protein is designated as preproenkephalin B, while adrenal preproenkephalin is referred to hereafter as preproenkephalin A.

Isolation of cDNA clones and strategy of DNA sequencing

The rationale for cloning DNA sequences complementary to preproenkephalin B mRNA was the same as that described for cloning cDNA for preproenkephalin A (ref. 8), except that we

constructed a library of cDNA clones using the plasmid DNA vector elaborated by Okayama and Berg¹⁴. This vector itself serves as the primer for first- and ultimately second-strand cDNA synthesis and thus facilitates the cloning of larger cDNA species. The cDNA library derived from porcine hypothalamic poly(A)-containing RNA was screened by hybridization with a mixture of eight oligodeoxyribonucleotides (Fig. 1), which were synthesized as two pools (a and b) by the modified triester method¹⁵. These tetradecamer pools represented all possible cDNA sequences predicted from the carboxy-terminal pentapeptide sequence of porcine dynorphin^{4,5}, Lys-Trp-Asp-Gln (excluding the third nucleotide residue of the Gln codon). Seventy-five hybridization-positive clones were isolated from about 250,000 transformants. Twelve of them were analysed with the restriction endonucleases *Ava*II, *Hin*I and *Dde*I and were classified into seven groups according to the restriction patterns obtained. Nucleotide sequence analysis by the Maxam-Gilbert procedure¹⁶ of appropriate restriction fragments that hybridized with the tetradecamer probes revealed that one group, including five clones, carried a DNA segment encoding the whole or a portion of the dynorphin sequence. Two clones of this group, pLEN47 (harbouring the largest cDNA insert) and pLEN21, were subjected to DNA sequencing¹⁶ according to the strategy indicated in Fig. 2 (see Fig. 2 legend for experimental details of cDNA cloning).

The five clones mentioned above, including pLEN47 and pLEN21, did not carry the entire protein-coding sequence. Moreover, none of the other clones isolated contained cDNA inserts extending as far as that of clone pLEN47 because they did not hybridize with the *Rsa*I(617)-*Alu*I(658) fragment (Fig. 2) derived from the 5'-terminal region of this clone as shown by *in situ* colony hybridization¹⁷. Therefore, a further attempt was made to clone DNA sequences complementary to the remaining portion of preproenkephalin B mRNA.

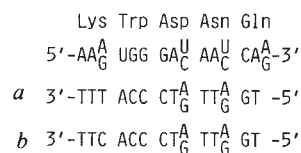
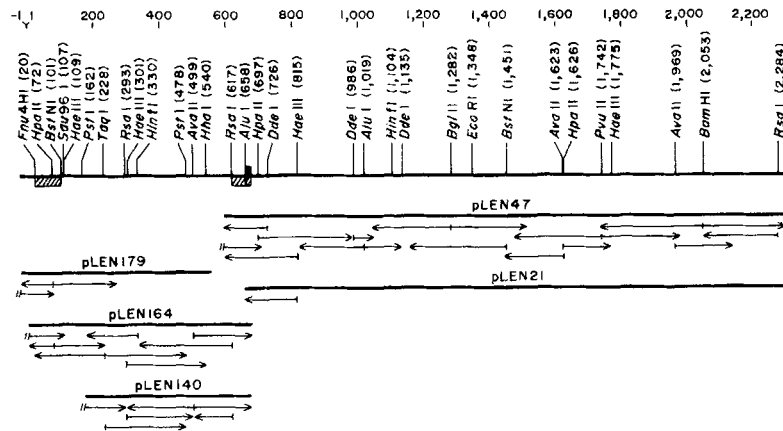


Fig. 1 Synthetic oligodeoxyribonucleotides used for probing cloned cDNA for preproenkephalin B and/or for priming the reverse transcription of preproenkephalin B mRNA. All possible coding sequences and the corresponding tetradecamers synthesized as two pools (a and b) are given for the carboxy-terminal pentapeptide sequence of porcine dynorphin (residues 221-225 of preproenkephalin B); for the numbering of amino acid residues, see Fig. 3.

Fig. 2 Strategy for sequencing the cDNA inserts in clones pLEN47, pLEN21, pLEN164, pLEN140 and pLEN179. For the isolation of clones pLEN47 and pLEN21, a cDNA library was constructed by the method of Okayama and Berg¹⁴ using 3.0 µg of poly(A) RNA from porcine hypothalamus and 5.6 µg of the vector-primer DNA; avian myeloblastosis virus reverse transcriptase was provided by Dr J. W. Beard. Poly(A) RNA was isolated by subjecting total RNA extracted²⁸ to oligo(dT)-cellulose chromatography²⁹ twice. *Escherichia coli* λ1776 (ref. 30) or HB101 (ref. 31) was used for transformation³², and ampicillin-resistant transformants were screened³³ by hybridization at 36 °C with an equimolar mixture of the two oligodeoxyribonucleotide pools (Fig. 1) labelled with ³²P at the 5' end. For the isolation of clones pLEN164, pLEN140 and pLEN179, single-stranded cDNA was synthesized using the oligodeoxyribonucleotide pool *b* as primer and porcine hypothalamic poly(A) RNA as template and was converted to double-stranded cDNA, which was cloned in plasmid pBR322 by inserting it into the *Pst*I site using poly(dG)·poly(dC) homopolymeric extensions; the procedures used were as described previously⁸. The cDNA clones obtained were screened³³ by hybridization at 45 °C (pLEN164 and pLEN140) or 60 °C (pLEN179) with the 5'-³²P-labelled restriction fragments specified in the text. The restriction map displays only relevant restriction endonuclease sites, which are identified by numbers indicating the 5'-terminal nucleotide generated by cleavage (for the nucleotide numbers, see Fig. 3). The poly(dA)·poly(dT) tract and the poly(dG)·poly(dC) tails are not included in the restriction map, except for the poly(dG)·poly(dC) tail at the 3' end of pLEN179; the location of this terminus is approximate. The sequence used for specific priming of reverse transcription is indicated by a closed box, and those used as hybridization probes for selecting clones by shaded boxes. DNA sequencing was carried out by the procedure of Maxam and Gilbert¹⁶. The direction and extent of sequence determinations are shown by horizontal arrows under each clone used; the sites of 5'-end labelling are indicated by short vertical lines at the end of arrows. The slash marks at the end of arrows mean that the site of 5'-end labelling was located on the vector DNA^{14,34,35} as follows (the numbers in parentheses indicate the distance in nucleotides between the site of labelling and the *Pst*I site flanking the cDNA insert): *Bst*NI (45) for pLEN47; *Sau*3AI (66) for pLEN164; *Ava*II (122) for pLEN140; *Ava*II (107) for pLEN179. Further details of the sequencing procedures have been described previously⁷.



Nucleotide sequence analysis of clones pLEN47 and pLEN21 revealed that the pentapeptide sequence Lys-Trp-Asp-Asn-Gln is encoded by the mRNA sequence 5'-AAGUGGGACAACCAG-3'. Therefore, one of the above-mentioned synthetic oligodeoxyribonucleotide pools (pool *b*), which contained the tetradecamer complementary to this mRNA sequence, was used for specific priming of the reverse transcription of preproenkephalin B mRNA. The resulting cDNA was cloned in plasmid pBR322 and screened by hybridization with the *Rsa*I(617)-*Alu*I(658) fragment (Fig. 2). Fifty-nine hybridization-positive clones were isolated from about 170,000 transformants. Two of these clones, pLEN164 and pLEN140, which carried larger cDNA inserts, were subjected to DNA sequencing¹⁶ (Fig. 2). The 3'-terminal regions of clones pLEN164 and pLEN140 contained sequences of 82 and 78 base pairs (bp), respectively, that overlapped the 5'-terminal sequence of clone pLEN47.

In an attempt to clone DNA sequences complementary to the 5'-terminal region of preproenkephalin B mRNA, we rescreened the cDNA clones obtained by the reverse transcription of the mRNA primed with the oligodeoxyribonucleotide pool *b*, using the *Fnu*4HI(20)-*Bst*NI(101) fragment (Fig. 2) derived from the 5'-terminal region of clone pLEN164 as a hybridization probe. Among the eight hybridization-positive clones isolated from about 200,000 transformants, clone pLEN179 contained the largest *Pst*I fragment (upstream from the *Pst*I site at position 162) that hybridized with the probe. This clone, subjected to DNA sequencing¹⁶ (Fig. 2), shared a sequence of at least 260 bp with clone pLEN164.

Nucleotide sequence of preproenkephalin B mRNA and assignment of protein sequence

The primary structure of porcine preproenkephalin B mRNA (Fig. 3) was deduced from the 2,333-nucleotide sequence (excluding the poly(dA)·poly(dT) tract and poly(dG)·poly(dC) tail) determined for the cDNA inserts of clones pLEN47, pLEN21, pLEN164, pLEN140 and pLEN179. The nucleotide sequence of the whole protein-coding region was determined with at least two clones. The sequences of nucleotide residues 523-549 and 625-675 correspond precisely to the amino acid sequences determined for β -neo-endorphin³ (amino acid residues 175-183) and dynorphin^{4,5} (amino acid residues 209-225), respectively; α -neo-endorphin¹⁸ is represented by amino acid residues 175-184. The sequence of nucleotide residues 682-696 encodes a third Leu-enkephalin sequence (amino acid

residues 228-232), the carboxyl end of which is connected by the paired basic residues Arg-Arg with a sequence of 22 amino acids. The carboxyl terminus of the latter sequence is followed by the translational termination codon UAA. The sequences of β -neo-endorphin and dynorphin as well as the three Leu-enkephalin sequences are each bounded by paired basic amino acid residues. Neither an enkephalin sequence nor a dibasic structure is present upstream of the Lys-Arg pair immediately preceding the β -neo-endorphin sequence. Five nucleotide differences are observed in the protein-coding region between the individual clones used for sequence determinations, as noted in Fig. 3 legend. Two of these differences result in amino acid replacements at position 147 (Gly or Glu) and position 197 (Arg or Gly), whereas the other differences lead to synonymous codons. The observed nucleotide differences may be due to polymorphism^{19,20} in the porcine preproenkephalin B gene because the template RNA used for *in vitro* cDNA synthesis was derived from the pooled hypothalami of many individuals. However, possible errors occurring during *in vitro* cDNA synthesis or DNA cloning²¹ cannot be excluded.

The translational initiation site was tentatively assigned to the methionine codon AUG at positions 1-3, which is the first AUG triplet within the mRNA sequence deduced, because the sequence of the 20 amino acid residues starting with this methionine (residue 1) exhibits a feature characteristic of the signal peptide present at the amino-terminal end of secretory proteins²²; it contains many hydrophobic amino acids (16 non-polar residues including six leucine residues). This assignment seems plausible because the size of the specific cDNA transcript formed by reverse transcriptase-mediated elongation of the *Fnu*4HI(20)-*Hpa*II(72) fragment (Fig. 2) derived from the 5'-terminal region of clone pLEN164 suggests that no more than ~150 nucleotides are missing from the cloned sequence shown in Fig. 3. This is also consistent with the size of porcine preproenkephalin B mRNA (~2,600 nucleotides) estimated by blot hybridization analysis of hypothalamic poly(A) RNA (Fig. 4).

The signal peptide of secretory proteins generally contains a region rich in hydrophobic amino acids with large side chains in its central portion and terminates in a residue with a small neutral side chain²³ (for example, alanine, glycine or serine). Therefore, a possible site for cleavage of the signal peptide of preproenkephalin B seems to be after the alanine residue at position 20. After removal of the signal peptide including the cysteine residue at position 11, the remaining prohormone would contain an even number (six) of cysteine residues, which are all located in its amino-terminal region and which may form

The structure of porcine proenkephalin B is schematically illustrated in Fig. 5. The precursor protein is composed of 256 amino acids including a putative signal peptide of 20 amino acids. The carboxy-terminal region of proenkephalin B contains the two known opioid peptides β -neo-enkephalin and dynorphin and a third Leu-enkephalin sequence with a carboxyl extension. Paired basic amino acid residues flank the structures of β -neo-

endorphin and dynorphin as well as the three Leu-enkephalin sequences. A survey of various prohormones suggests that the Lys-Arg pair may represent a primary site of proteolytic processing as discussed previously^{8,23}. If this is valid for proenkephalin B, the primary Leu-enkephalin-containing products formed from it would be β -neo-endorphin, dynorphin and the peptide composed of its carboxy-terminal 29 amino acid residues (residues 228–256). It is tempting to assume that this carboxy-terminal peptide represents a novel opioid peptide

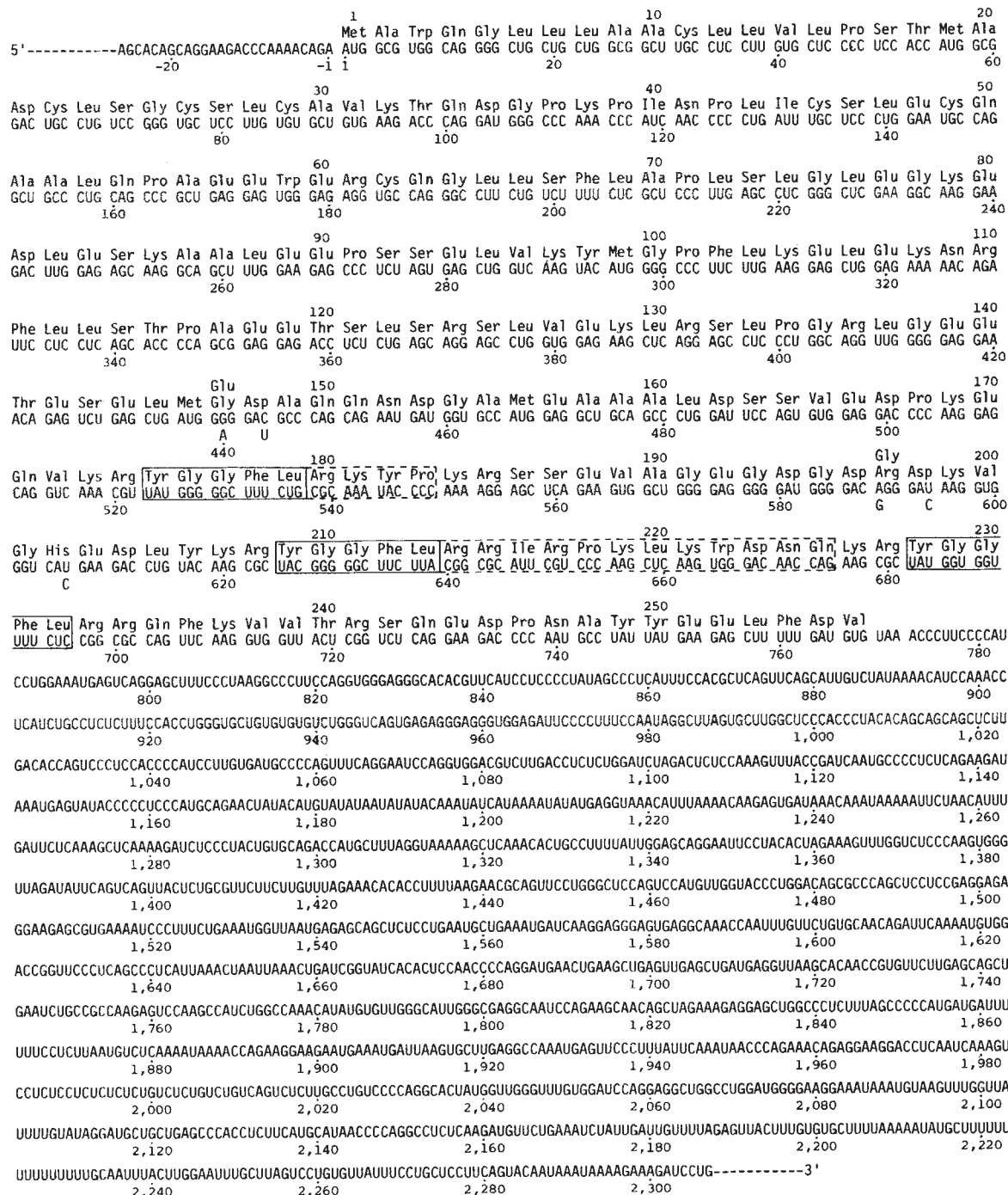


Fig. 3 Primary structure of porcine preproenkephalin B mRNA. The nucleotide sequence of the mRNA was deduced from those of the cDNA inserts in the clones given in Fig. 2. Nucleotide residues are numbered in the 5' to 3' direction, beginning with the first residue of the AUG triplet encoding the initiative methionine, and the nucleotides on the 5' side of residue 1 are indicated by negative numbers. The 5'-terminal sequence presented does not extend to the 5' end of the mRNA (see the text), whereas the 3'-terminal sequence shown is followed by a poly(A) tract of ~120 nucleotides connected with the vector DNA sequence^{34,35}, thus representing the complete sequence of this region. The predicted amino acid sequence is displayed above the nucleotide sequence, and amino acid residues are numbered beginning with the initiative methionine. The Leu-enkephalin sequences are boxed with solid lines, and their carboxyl extensions in β -neo-enkephalin and dynorphin with dashed lines. The nucleotide differences in the protein-coding region observed between the individual clones used are as follows: G (pLEN164) or A (pLEN140) at position 440; C (pLEN164) or U (pLEN140) at position 444; A (pLEN164) or G (pLEN140) at position 589; U (pLEN164 and pLEN140) or C (pLEN47) at position 594; U (pLEN47 and pLEN140) or C (pLEN164) at position 606. The nucleotide differences at positions 440 and 589 result in the replacements of Glv at position 147 and Arg at position 197 by Glu and Gly, respectively.

because it exhibits a structural similarity to β -neo-endorphin and dynorphin in that they all contain, at their amino-terminal end, a Leu-enkephalin sequence which is connected by paired basic residues, Arg-Arg or Arg-Lys, with a unique peptide sequence. It is also possible that these Leu-enkephalin-containing peptides undergo further proteolytic cleavage to yield smaller opioid peptides including Leu-enkephalin itself.

All the structures of endogenous opioid peptides thus far documented can now be identified as a partial sequence of one or more of the three precursor proteins preproenkephalin B, preproenkephalin A and the ACTH/ β -LPH precursor. The three opioid peptide precursors exhibit similar structural organizations in that they all have multiple repetitive units in the carboxy-terminal one- or two-thirds of their molecules and a cysteine-containing amino-terminal sequence preceded by a signal peptide^{7,8,12,13}. It is remarkable that the six cysteine residues in the amino-terminal regions of proenkephalin B and proenkephalin A are located at equivalent positions after the outative cleavage site of the signal peptide, except that the distance between the third and fourth residues differs by one amino acid residue. Moreover, the amino-terminal regions of the two prohormones share several additional amino acids at equivalent positions. The observed homology in the amino-terminal region suggests that a proper folding of this region by disulphide bonds may be involved in the processing of these orohormones or in the function of the peptides derived from this region. Many opioid peptides contain a Met-enkephalin or Leu-enkephalin sequence at their amino-terminal end, and the peptide sequence following the enkephalin structure may modify their physiological activity by conferring additional specificity for recognition of cell-surface receptors, by increasing the binding affinity or by affecting their stability *in vivo*. Thus, preproenkephalin B, like the ACTH/ β -LPH precursor and preproenkephalin A, may represent a multi-hormone precursor^{7,8,25} from which multiple biologically active peptides are elaborated by proteolytic processing to perform coordinate functions in the central nervous system as well as in peripheral tissues.

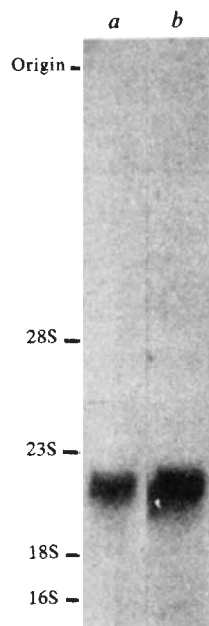


Fig. 4 Autoradiogram of blot hybridization analysis of hypothalamic RNA with cDNA probes. Porcine hypothalamic poly(A) RNA (30 μ g) was denatured with 1 M glyoxal and 50% dimethyl sulphoxide³⁶, electrophoresed on 1.5% agarose gel and transferred to diazobenzoyloxymethyl paper³⁷. The hybridization probe used was the 316-bp *Pst*I fragment (residues 162–477) derived from clone pLEN164 (a) or the 1,441-bp *Hind*III–*Pvu*II fragment containing a cDNA sequence of 1,149 bp (residues 593–1,741) derived from clone pLEN47 (b); both probes were labelled by nick-translation³⁸ with [α -³²P]dCTP. The size markers used were porcine and *E. coli* rRNA³⁶.

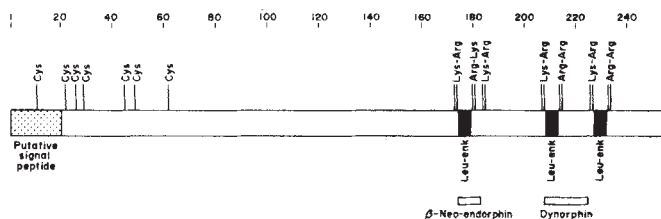


Fig. 5 Schematic representation of the structure of porcine preproenkephalin B. The Leu-enkephalin sequences are indicated by closed boxes, and the putative signal peptide by a stippled box. The positions of β -neo-endorphin and dynorphin are shown underneath by open bars. All the paired basic amino acid residues and cysteine residues are displayed. Amino acid numbers (see Fig. 3) are given above the structure.

Characteristic features of preproenkephalin B mRNA

Computer analysis of the nucleotide sequence data presented in Fig. 3 shows that preproenkephalin B mRNA contains, in addition to the coding regions for the three repeated Leu-enkephalin sequences, many segments that seem to represent direct duplications (for example, residues 6–21 and 183–198; 407–421 and 2,071–2,085; 455–470 and 1,464–1,479; 604–619 and 1,163–1,178; 683–697 and 1,854–1,868; 847–862 and 1,840–1,855; 1,135–1,150 and 1,200–1,215; 1,398–1,414 and 2,189–2,205). This suggests that the structural gene for preproenkephalin B has evolved by a series of direct duplications of certain primordial DNA segments. Similar observations were made for the mRNAs encoding the ACTH/ β -LPH precursor⁷ and preproenkephalin A (ref. 8), other multi-hormone precursors containing multiple repetitive units. Codon utilization for preproenkephalin B mRNA occurs nonrandomly, generally reflecting a preference for G or C in the third position of the codons, as noted for other eukaryotic mRNAs²⁶.

A peculiar feature of preproenkephalin B mRNA is its unusually long 3'-untranslated sequence (1,539 nucleotides excluding the poly(A) tract). This does not seem to result from either possible errors occurring during *in vitro* cDNA synthesis or DNA cloning, because all the five cDNA clones analysed that carry the 3'-untranslated region exhibit common restriction endonuclease sites and because the size of the mRNA estimated by blot hybridization analysis is sufficiently large to account for the length of this region (Fig. 4). Furthermore, the 3'-untranslated region of the mRNA contains 75 in- and out-of-frame nonsense codons and no open translational reading frame longer than 213 nucleotides. It also contains five copies of the sequence AAUAAA (residues 1,245–1,250, 1,883–1,888, 2,084–2,089, 2,286–2,291 and 2,290–2,295), two of which overlap and are located 13 and 17 nucleotides upstream from the poly(A) tract, probably being involved in poly(A) addition after transcription²⁷. Despite the presence of the other AAUAAA sequences further upstream, shorter mRNA species were not detected by blot hybridization analysis (Fig. 4).

We thank Dr Paul Berg and Dr Hiroto Okayama for providing us with their high-efficiency cloning system before its publication, and Dr Tatsuo Ooi for his help in computer analysis of the nucleotide sequence. This investigation was supported in part by research grants from the Ministry of Education, Science and Culture of Japan, the Mitsubishi Foundation and the Japanese Foundation of Metabolism and Diseases.

Received 26 March; accepted 10 May 1982.

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LETTERS TO NATURE

Limits on the nucleus of Halley's Comet

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We report here a limiting magnitude to the brightness of the nucleus of Halley's Comet which, together with published interpretations of the comet's visual light curve in 1910 and estimates of the magnitude of non-gravitational forces that have modified the comet's orbital motion throughout the past five apparitions, places some interesting limits on the physical properties of the nucleus. We find that the nucleus is probably ~4 km across, has a surface albedo of 0.17, and a mass of 3.4×10^{16} g. Comet Halley may not be recovered until early November 1984, but could be found as early as late September–early October 1982.

During the 1981–82 observing season we have been able to place the following limit on the total brightness of Comet Halley in the photometric V band. Assuming that the nucleus has a stellar-like appearance we find

$$V \geq 24.3 \quad (1)$$

This limit is based on observations at the Kitt Peak National Observatory (KPNO) on the night of 2–3 December, 1981 with the 4-m Mayall telescope and the KPNO video camera. The total observing time during which usable data was collected was 180 min with the mid-time falling at 3.37 UT December 1981. According to the ephemeris of D. Yeomans (personal communication) the comet was at a distance of 12.81 AU from the Sun and 12.03 AU from the Earth. At the time it was thought that the nuclear brightness might be as bright as 24.0 mag. The phase angle at the time of observation was 2.8°.

On this night the atmosphere was quite clear with good seeing. The full width at half intensity of a star image was measured at 1.6 arc s. The video camera has a square field of view, ~60 arc s per side, and the pixels of the image are roughly 0.28 arc s across. The camera works in what is essentially a photon counting mode for faint objects by co-adding successive short exposures on an intensified silicon vidicon into a frame buffer. The observations were made in a broad visual (not V) passband through a KG-3 filter which suppressed OH emission in the sky background. The data set consists of 20 separate 9-min exposures guided at the diurnal rate. These exposures were added together in a way which cancels the apparent motion of the comet to maximize its visibility. The V calibration was obtained through observations, on the same night, of 17 stars in a standard field of NGC2419 (ref. 1). Although the cluster contains few stars with colours close to that of the Sun we

estimate that our limit is good to 0.1 mag. Extinction corrections were made with mean KPNO coefficients.

Limiting magnitudes were estimated in two ways. Inequality (1) represents the fact that there is no indication of the comet in the data at the level of one standard deviation above the mean level of the sky integrated over areas 4.2 arc s in diameter, that is, 2.6 times the FWHM of the seeing function. The statistics of the sky were determined through measurements of 11 apparently blank areas not thought to be contaminated by stellar or other faint objects.

In a second approach, the magnitudes of five barely discernible (even under a powerful stretch with the image processor) objects in the summed, and unshifted, data were calculated. These objects yielded mean V magnitudes of 24.0.

Before exploring the implications of inequality (1) we note that: (1) the comet is almost certainly somewhere in the 60×60 arc s field. Yeomans believes his ephemeris to be good to ~ 10 arc s⁻¹. The line of variation along the comet's orbit was inclined to the predicted track of the comet by only a small angle ($\sim 20^\circ$), and an unlikely error of 1 day in the time of perihelion passage will displace it by only 20 arc s from its predicted position. Other positional errors are minor by comparison. (2) The probability that the comet is accidentally hidden under a star trail can be estimated from the relative area of the trails in the picture. (The comet's apparent motion is quite large, ~ 11 arc s h⁻¹.) We find the probability of this happening to be ~ 0.28 , which is sizeable but not hopeless. This problem is probably the severest of those faced by the observer and calls for careful pre-selection of the date and time of observation. (3) Several other, independent, attempts to recover the comet during the 1981–82 opposition have been informally reported to us. Limiting magnitudes as faint as 25.5 have been mentioned but it is unknown how well these estimates are calibrated or whether they are V magnitudes. (4) The results of the study by Yeomans² on the comet's orbital motion demonstrate that 'non-gravitational' forces are both measurable and have remained roughly constant in time for many past orbits. Moreover, the relative magnitudes of the radial and transverse components indicate that the nucleus rotates slowly and that the scale length over which these forces operate has the value (2.8 AU) expected for a nucleus whose primary volatile is water (see ref. 3). The coefficient A_1 of the radial component has a recommended (D. K. Yeomans personal communication) value of 0.28×10^{-8} AU (Ephemeris day)⁻². (5) By comparing the 1910 light curve for Comet Halley with those of more recent comets for which UV observations are available, Newburn^{4,5} has estimated the total production rate of molecules, Q s⁻¹ near 1 AU for Comet Halley. Pre-perihelion he finds $Q \sim 1.8 \times 10^{29}$ s⁻¹ and post-perihelion $\sim 3.2 \times 10^{29}$ s⁻¹. On the basis of these results we assume that the production rate at 1 AU definitely lies in the range $10^{28} < Q < 10^{30}$ s⁻¹ and that 2×10^{29} s⁻¹ is the preferred value.

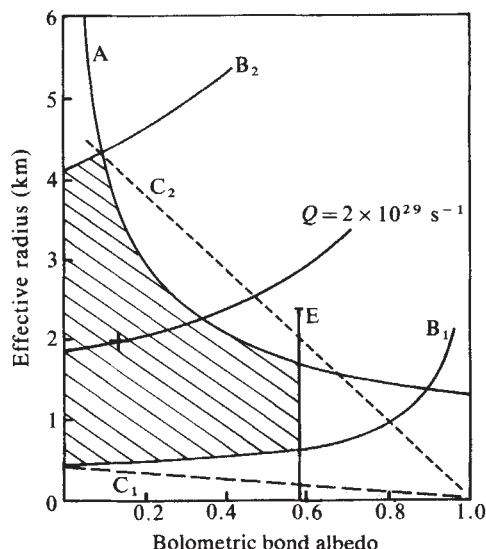


Fig. 1 The cross-hatched area shows the region in which the effective radius and albedo of the nucleus of Halley's comet probably lies. The area is bounded by the following curves: A, set by the visual magnitude limit; B₂ and B₁, set by upper (10^{30} s^{-1}) and lower (10^{28} s^{-1}) bounds on the value of Q at 1 AU; E, the albedo of Europa's fresh, icy surface. The curves C₁ and C₂ indicate the limiting relationships between effective radius, effective density and albedo. The curve $Q = 2 \times 10^{29} \text{ s}^{-1}$ represent the locus of points near which Halley's nucleus is most likely to fall, and the vertical cross indicates the position of the comet if $\alpha = 1$ and $\bar{\rho} = 1 \text{ g cm}^{-3}$ are included as an extra constraint.

We now proceed to an analysis of the above material on the basis of the following physical model. We assume that the nucleus is a non-rotating, water ice, sphere characterized by a radius R (cm), a bolometric Bond albedo A , and a mean density $\bar{\rho}$ (g cm^{-3}). As the surface cannot be expected to be uniformly active we assume that a fraction, $(1 - \alpha^2)$, of the nucleus neither scatters light nor is involved in the production (by sublimation) of water molecules. Thus surface 'activity', and all effects related to it, are assumed capable of representation by a single parameter, α . Finally we choose to consider two very different models for the interaction of the nucleus with light. Case A: its surface acts like that of the satellite Europa which is thought to be relatively fresh ice. Europa has a high albedo. Case B: the surface behaves like that of Callisto which is an ancient icy surface of low albedo⁶.

Denoting $V(1, 0)$ as the magnitude of the nucleus at 1 AU and zero phase angle, and $p(V)$ as the geometric albedo of the fraction of the surface that scatters light, we have

$$V(1, 0) = -2.5 [\log(p(V)(\alpha R)^2) + \log C] \\ = V - 5 \log(rd) - \Delta m(a) \quad (2)$$

Where C is a constant, V the observed magnitude, r and d are the solar and geocentric distances at the time of observation, and $\Delta m(a)$ is the phase correction at phase angle a . Using photometric data for Callisto and Europa we find that our limit on V implies that: case A: $p(V)(\alpha R)^2 \leq 1.91 \times 10^{10} \text{ cm}^2$, $\log C = -15.594$, $\Delta m = 0.084$, and $V(1, 0) \geq 13.28 \text{ mag}$. Case B: $p(V)(\alpha R)^2 \leq 2.23 \times 10^{10} \text{ cm}^2$, $\log C = -15.606$, $\Delta m = 0.22$, and $V(1, 0) \geq 13.14 \text{ mag}$.

We now adopt the techniques developed by Delsemme and Rudd⁷ and Whipple⁸. To find the relationship between $p(V)$ and A we note that for Europa $p(V) = 0.68$, $A/p(V) = 0.85$; and for Callisto $p(V) = 0.17$, $A/p(V) = 0.76$ (ref. 9). Thus for both cases A and B our V magnitude limit places essentially the same photometric limit on $A(\alpha R)^2$, that is,

$$A(\alpha R)^2 \leq 1.69 \times 10^{10} \text{ cm}^2$$

Further restriction can be placed on R and A through the

measured value of A_1 and the limits on Q at 1 AU. From ref. 7 we find that at 1 AU

$$(1 - A)(\alpha R)^2 = 1.71 \times 10^{19} Q \text{ cm}^2$$

and from ref. 8 the radial non-gravitational force due to the vaporization of water ice at 1 AU is approximately

$$F_r = 5.8(1 - A)(\alpha R)^2 \text{ dynes}$$

The exact value of the force is uncertain, partly because of the lack of definite knowledge on the velocity of the escaping gases and partly due to our ignorance of the precise geometry of the outflow. Yeoman's² results on non-gravitational forces imply a radial acceleration of $\sim 5.6 \times 10^{-6} \text{ cm s}^{-2}$ at 1 AU and so from Newton's third law we find that the mass of the nucleus is given by

$$M = \frac{4\pi}{3} \bar{\rho} R^3 = 1.0 \times 10^6 (1 - A)(\alpha R)^2 \text{ g}$$

Writing $\bar{\rho}' = \bar{\rho}\alpha^{-3}$ (an 'effective' density) and $R' = \alpha R$ (an 'effective' radius) we derive the following three, independent, constraints on the physical properties of Halley's nucleus:

$$A(R')^2 \leq 1.69 \text{ km}^2 \quad (A)$$

$$0.17 \leq (1 - A)(R')^2 \leq 17.1 \text{ km}^2 \quad (B)$$

$$R'/(1 - A) = 2.4/\bar{\rho}' \text{ km} \quad (C)$$

In these expressions R' is in km and $\bar{\rho}'$ is in g cm^{-3} . Two further constraints that we find useful to introduce at this point are first, the bolometric bond albedo of the nucleus is most likely to be less than that of the fresh icy surface of Europa ($A = 0.58$), and second, the mean density of the small, uncompressed nucleus is likely to be less than that of Callisto ($\bar{\rho} = 1.79 \text{ g cm}^{-3}$).

In Fig. 1 we explore these constraints in the $(R' - A)$ plane and find that they clearly limit the domain in which the effective radius and effective density must lie. Analysis shows that

$$0.41 \leq R' \leq 4.3 \text{ km}$$

$$0.50 \leq \bar{\rho}' \leq 5.9 \text{ g cm}^{-3}$$

$$1.7 \times 10^{15} \leq M \leq 1.9 \times 10^{17} \text{ g}$$

The constraint on the mass, M , comes directly from the limits on Q and the value of A_1 . Because of the way in which we have parameterized surface 'activity', the limits on mass are independent of the activity factor α .

With the assumed upper limit of 1.79 g cm^{-3} for the mean density and the lower limit to the mass we find a lower limit to the physical radius of 0.61 km. An upper limit to the radius, or lower limit to the mean density cannot be estimated without more assumptions.

To provide further insight into the possible range of size for Halley we assert that the material of the nucleus is likely to be compacted and that the mean density must be near 1 g cm^{-3} . This is based simply on the expectation that the nucleus is primarily water ice that has agglomerated from smaller units that collided and stuck together at velocities large enough to compact the material. If this is true then it follows that

$$0.77 < R < 3.5 \text{ km}$$

and

$$0.56 < \alpha \leq 1$$

The range is further narrowed if we assert that $Q = 2 \times 10^{29} \text{ s}^{-1}$, that is, the most likely value, at 1 AU. If this is true then $M = 3.4 \times 10^{16} \text{ g}$, $R = 2.0 \text{ km}$, $0 < A < 0.17$, and $0.95 < \alpha < 1$. We believe that these values are the best estimates that can be made at present of the parameters that characterize Halley's nucleus. The chief uncertainty comes from the assumption of the value for the mean density which could be substantially lower if the nucleus is less compacted than has been assumed here.

Because the lower limit to the albedo could, at least in principle, be zero it is impossible to predict a minimum bright-

ness for the comet and thus confidently estimate the latest time at which the nucleus might be recovered. However, if we consider a geometric albedo of 0.02 to be a reasonable lower limit on the basis of our experience with asteroids⁹, then $V(1, 0)$ might be as large as 16.88 mag. If this is the case then the video camera at the 4-m Mayall telescope cannot be expected to reach the comet until its heliocentric distance is reduced to ~5.8 AU in early November 1984.

Based on the above estimates we also believe that the comet nucleus could probably be as bright as 14.14 mag, in which case it was at 24.7 mag, barely half a magnitude below our limit, in December 1981. If the nucleus is indeed this bright then recovery can be expected in late September–early October 1982.

Operated by the Association of Universities for research in Astronomy, Inc., under contract to the NSF.

Received 25 February; accepted 2 June 1982.

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Evidence from Sr isotopes for long-lived heterogeneities in the upper mantle

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A recent Rb–Sr study of selected suites of samples, mainly from Precambrian alkaline rocks of northern Ontario, may have some bearing on mantle differentiation and evolution of the continental crust. We show here that initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios from most of the complexes, plotted as a function of age, define a straight line, and imply a source region for the Sr with a Rb–Sr ratio of 0.018 ± 0.002 that has behaved as a closed system for at least 2,600 Myr. Furthermore, the intersection of this line with the development line for 'bulk Earth' at about 2,800 Myr can be interpreted as evidence for a mean time of differentiation of bulk Earth material into an enriched crust and a residual, depleted mantle.

Figure 1 shows the distribution of some Precambrian alkaline complexes that together form one of the oldest and largest areas of alkaline magmatism yet documented; the carbonatites alone are found over an area of $1.3 \times 10^6 \text{ km}^2$ (ref. 1). Most of the complexes were intruded into the Superior Province during the middle and late Proterozoic², and form a series of unmetamorphosed, circular or tear-shaped plutons, mainly of miaskitic affinity. Their distribution seems to have been, in part, controlled by large-scale, structural features. The clustering around the Kapuskasing gravity and magnetic high is one such example.

We have recently obtained some whole-rock Rb–Sr isochron ages, and initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of high precision, from several of the complexes found within the Superior Province; these are summarized in Table 1. Also included are some previously published K–Ar ages^{2,3}, determined on minerals (mostly biotites) from the same complexes. In all cases, other than that of Township 107, the Rb–Sr ages seem to agree with the K–Ar

results (although no uncertainties are quoted for some of the latter they are probably of the order of $\pm 3\%$ of the quoted value). Samples from Big Beaver House, Firesand, Poohbah and Seabrook have Rb–Sr ratios that make them unsuitable for dating, so the age assigned to the complex is the K–Ar age. Their initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios were measured to high precision on whole-rock material enriched in Sr.

That the sub-oceanic mantle is chemically heterogeneous has now been established, and it seems that such heterogeneities must have persisted over a considerable period of time^{4–7}, at least ~2,000 Myr. Whereas the data from sub-oceanic rocks are quite convincing, the possibility of contamination by crustal assimilation complicates the interpretation of data from continental areas. Basic igneous rocks or their separated mineral phases have been used to monitor sub-continental mantle, but carbonatites and allied rocks are ideal for the same purpose because of their deep-seated origin (>100 km), their rapid movement to the surface, and Sr contents so high that even if significant contamination were to occur the carbonatites should still retain their original isotopic composition.

The data listed in Table 1 can be used to define a development line for the Sr source region from the late Archaean to the late Proterozoic. The isotopic data from all 13 complexes are plotted on a graph of age against initial ratio (Fig. 2). Of the 13 data points, 9 fall on a straight line and the good linear fit of the data (mean square of the weighted deviates⁸ = 1.8) implies that the source region of the Sr has behaved as a closed or near closed system with respect to the exchange of Rb and Sr between ~2,700 and 1,000 Myr, an interval of at least 1,700 Myr. The slope of the line yields a Rb/Sr ratio for the source region of 0.017 ± 0.002 (all uncertainties, 2σ , are calculated by the method of York⁹). This figure, similar to values proposed by Moor bath^{10,11} and earlier workers, is significantly different from the estimate of 0.03 for bulk Earth inferred by DePaolo and Wasserburg⁶ and O'Nions *et al.*¹² from Sm–Nd data, and the range of 0.025–0.035 ('Main path') suggested by Jahn and Nyquist¹³ and Jahn *et al.*¹⁴ as the source region of alkaline basalts and many Precambrian rocks. The extrapolated ratio for $t = 4,550 \text{ Myr}$ is 0.7000 ± 0.0004 , a value that is significantly higher than those proposed for meteoritic and lunar material, all of which are less than 0.69910 (refs 15–18). For $t = 0 \text{ Myr}$

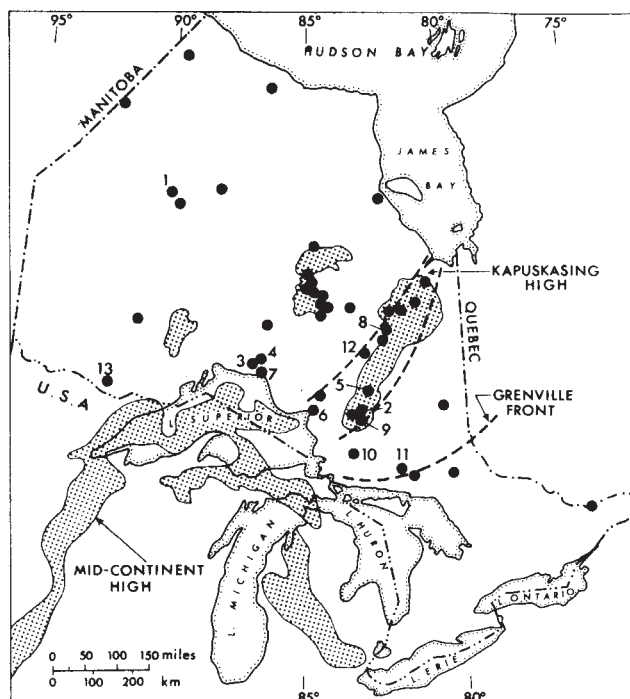


Fig. 1 Distribution of alkaline complexes of Ontario (after ref. 1). The numbers are identified on Table 1. Stippled areas are gravity highs.

Table 1 Rb–Sr ages and initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of alkaline complexes from Ontario, Canada

Complex	Details	Age* (Myr)	$R_i^\dagger$
1. Big Beaver House	Carbonatite dyke; also serpentinized pyroxenite	(1,015)	$0.70235 \pm 0.00010^\ddagger$
2. Nemegosenda Lake	Elliptical mass; central core—nepheline syenite, also jacupirangite, carbonatite + fenites	$1,015 \pm 65$ (1,020)	0.7038 ± 0.0004
3. Prairie Lake	Circular complex; central core—ijolite + minor nepheline syenite; thin shell of calcitic carbonatite	$1,030 \pm 70$ (1,120)	0.7025 ± 0.0003
4. Killala Lake	Circular, funnel-shaped body; mainly syenite, some gabbro	$1,045 \pm 30$ (1,195)	0.7038 ± 0.0006
5. Shenango	Tear-shaped body; mainly syenite + alkaline diorite	$1,045 \pm 14$ (1,085)	0.7036 ± 0.0004
6. Firesand River	Circular complex; abundant carbonatite, some mafic silicate rocks	(1,060)	$0.70249 \pm 0.00004^\ddagger$
7. Port Coldwell	Funnel-like mass; mainly syenite, some gabbro	$1,069 \pm 15$ (1,020)	0.7035 ± 0.0006
8. Clay-Howells	Dyke-like mass; mainly of carbonatite; near-circular mass of syenite	$1,075 \pm 15$ (1,020)	0.7028 ± 0.0005
9. Lackner Lake	Circular complex; ijolite and syenite, minor carbonatite	$1,095 \pm 30$ (1,100)	0.7028 ± 0.0004
10. Seabrook Lake	Tear-shaped body; carbonatite, ijolite, alkaline pyroxenite	(1,110)	$0.70240 \pm 0.00005^\ddagger$
11. Township 107 (Spanish River)	Dyke-like mass; carbonatite	$1,835 \pm 90$ (1,565)	0.7018 ± 0.0004
12. Cargill	Dumb-bell shaped body; carbonatite, with associated pyroxenite and possibly gabbro	(1,745)	$0.70200 \pm 0.00005^\ddagger$
13. Poohbah Lake	Funnel-like mass; mainly syenite	(2,680)	$0.70123 \pm 0.00010^\ddagger$

All errors given at 2σ level; $\lambda_{\text{Rb}} = 1.42 \times 10^{-11} \text{ yr}^{-1}$. Details after ref. 27.

* K–Ar ages^{2,3} in brackets; recalculated to current decay constants.

† $^{87}\text{Sr}/^{86}\text{Sr}$ ratios normalized to value of 0.70800 for Eimer and Amend.

‡ Initial ratio measured directly.

(the present) the extrapolated ratio is 0.70321 ± 0.00015 , a value comparable with that observed for alkaline basalts from oceanic islands^{12,19}. We note that Basu and Tatsumoto²⁰ have suggested, on the basis of Sm–Nd data, that carbonatites and oceanic island alkaline basalts have a common source.

Most of the samples that define the linear development line in Fig. 2 are from complexes with abundant carbonatite, and nearly all contain at least 3,000 p.p.m. Sr. The grouping of the data at ~1,000 Myr into two groups, A and B (see Fig. 2), however, requires further explanation. Group A includes the data from Nemegosenda, Killala, Port Coldwell and Shenango, and these seem to fall close to or on the bulk Earth development line; group B falls below. Three of the group A bodies are petrologically quite different from any of the younger complexes we have analysed. They are miaskitic in character, no carbonatite has been found associated with them, and samples from them have fairly low Sr contents (~500 p.p.m.). Nemegosenda, the fourth complex, is also miaskitic but it is associated with carbonatite enriched in Sr (~12,000 p.p.m.). Although the high initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios from these complexes could reflect crustal contamination during magma migration, the lack of any correlation between Sr isotopic ratio and Sr concentration, as well as the clustering of data into only two groups, suggests that the Sr isotopic composition of each group may well reflect the isotopic signature of its source region. Poohbah, the oldest complex shown in Table 1, also consists predominantly of miaskitic syenite; however, we have used data from it in the computation of the linear development line. Samples from Poohbah are high in Sr, unlike those from the 1,000-Myr group, and the initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.70123 ± 0.00010 is similar to the value of 0.70114 ± 0.00013 obtained by Hart and Brooks²¹ from 2,700 Myr clinopyroxenes from the Abitibi Belt, Superior Province, a value thought to reflect the Sr isotopic composition of the mantle at that time.

The $^{87}\text{Sr}/^{86}\text{Sr}$ value for the Earth at 4,550 Myr should be the same as that of some meteorites and lunar material, so the fact that our extrapolated $^{87}\text{Sr}/^{86}\text{Sr}$ ratio at 4,550 Myr is significantly higher indicates at least a two-stage history for the Sr source. Rather than interpreting the value as evidence that the Earth had a higher Rb/Sr ratio very early in its history²¹, we have looked instead at the intersection of the development line with that for bulk Earth^{6,12,22} which is equivalent to estimating the mean age of the reservoir²³ that yielded the Sr in the car-

bonatites and related rocks. The value obtained, about 2,800–3,100 Myr depending on the estimate of bulk Earth used, lies surprisingly close to the range of ages reported for granitoid and related rocks from a large part of the Canadian Shield, and to the time of separation of Superior Province rocks from the mantle²⁴. We propose that primitive mantle, similar in chemical composition to bulk Earth, underwent significant differentiation that culminated ~2,800 Myr ago in continental crust and a residual mantle that we would identify with the carbonatite Sr source. The data suggest that from at least 2,800 to 1,000 Myr the depleted mantle, overall, retained its isotopic identity and that within the precision of our measurements a linear evolution best describes the variation in $^{87}\text{Sr}/^{86}\text{Sr}$ ratio over this period of time. That the development line is so linear implies that the amount of sialic production from this reservoir has been relatively insignificant during post-Archaeon time.

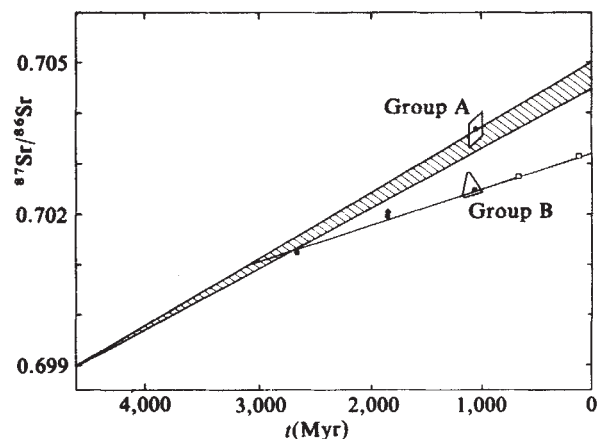


Fig. 2 $^{87}\text{Sr}/^{86}\text{Sr}$ growth lines for bulk Earth^{6,12,22} are contained within hatched envelope; the line shown is the best estimate for the depleted mantle source, using data from complexes intruded into the Superior Province (●) and Grenville Province (□). The least-square-fit line was calculated using a reproducibility of 90.00007 at the 1σ level for the high precision analyses rather than uncertainties based on internal precision. Centroids are shown for both the group A (four in all) and group B samples (six in all).

The addition of data points (see Fig. 2) from two much younger carbonatites that lie to the east in Grenville Province rocks changes the picture only slightly. Results from the St-Honoré (age ~650 Myr (ref. 25), $R_i = 0.70276$) and the Oka (age ~120 Myr (ref. 26), $R_i = 0.70318$) complexes fall on the extrapolation of the development line, and combined with the data from the Precambrian complexes yield a Rb/Sr ratio for the source of 0.018 ± 0.002 .

On the basis of the carbonatite data alone the depleted zone lies under at least parts of the Superior and Grenville Provinces, although its lateral extent may be considerably greater than we have demonstrated. The similarity of the present-day $^{87}\text{Sr}/^{86}\text{Sr}$ value of the depleted source (0.7032) with the range of values from young island-arc volcanics, coastal batholiths and some oceanic island basalts has not escaped our attention, and such an observation suggests that this zone may underlie both oceanic and continental areas. However, we note that if this zone is present in oceanic environments it is quite different from the mantle that is currently generating basalts at mid-ocean ridges.

We thank R. P. Sage for constant help, encouragement and enthusiasm during this project, and D. H. Watkinson for helpful discussions. This work was supported by the Ontario Geological Survey of the Ministry of Natural Resources (grant 42).

Received 7 August 1981; accepted 17 May 1982.

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New light on the equatorial source of pulsating aurora

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In recent years considerable circumstantial evidence has amassed to suggest that equatorial pulsating ELF hiss (100–600 Hz) is directly responsible for precipitation of the energetic electrons that produce pulsating aurora. Here we present the first evidence showing that the ELF hiss at the equator of the auroral geomagnetic field is directly related to pitch angle scattering of electrons >22 keV into the loss cone with subsequent precipitation into the auroral atmosphere.

During an auroral substorm one or more auroral arcs break up into many luminous patches which independently pulsate with periods in the range 2–20 s. At this time precipitating electrons (2–40 keV) measured by rockets at altitudes above 80 km are also found to be similarly modulated as are the bremsstrahlung X rays produced by these electrons in the atmosphere and measured by balloons around 30 km altitude (see ref. 1 for more details of these measurements).

One important result of these earlier measurements was the deduction^{2,3} of an equatorial location for the source of the modulations from the velocity dispersion of the pulsations observed in the energetic electrons at rocket altitudes. The only relevant theory to date for such a source is that of Coriniti and Kennel⁴ who argue that Whistler-mode waves are responsible for the pitch angle scattering of electrons >10 keV in the equatorial diffusion region. They showed that small amplitude micropulsations in the Earth's magnetic field can significantly modulate the Whistler mode wave growth and hence the electron precipitation flux. However, this theory requires simultaneous micropulsations which have yet to be observed at these times.

The European Space Agency spacecraft GEOS 1 and GEOS 2 are ideally instrumented⁵ and well placed for studies of this equatorial diffusion region. In particular GEOS 2 is positioned at the equator of field lines mapping down to the area of the Scandinavian observatories. Pulsating electromagnetic ELF hiss with periods in the range 2–20 s was found in GEOS 1 and 2 to follow auroral substorms, occurred at the same local times as pulsating aurora and showed a similar variation with the geomagnetic activity index, K_p (refs 6, 7). Furthermore pulsating ELF was generally observed when pulsating aurora was taking place at the foot of the GEOS field line^{6,7}.

Another piece of evidence relating the pulsating ELF hiss to pulsating aurora lies in the well known '3-Hz' oscillation sometimes observed optically during the pulsation⁸. Recently two independent rocket flights^{9,10} have observed 3-Hz oscillations in precipitating particles at the same time as the equatorial ELF hiss seen by GEOS 2 was pulsating and also simultaneously modulated with a similar frequency (~2.5 Hz). However, the 3-Hz frequencies did not match precisely at the equator and at the rockets but it would be unlikely that the spacecraft was on the exact same field line as the rockets.

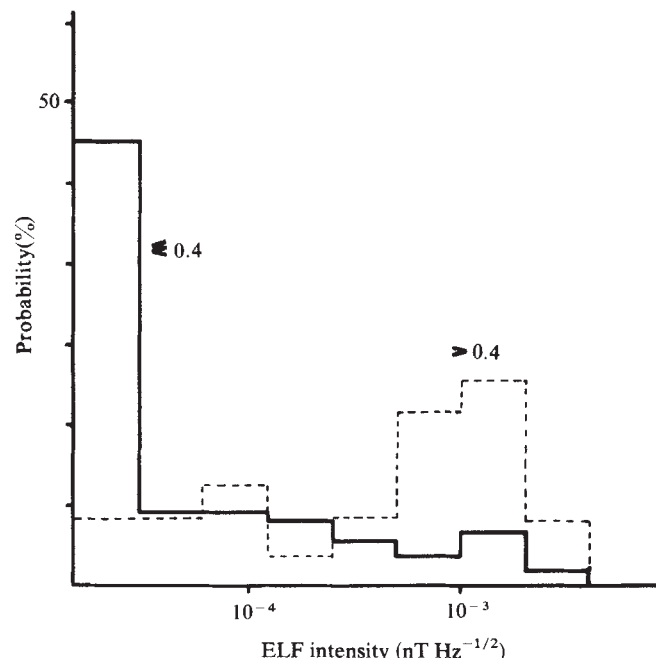


Fig. 1 Normalized histograms of occurrence of ELF wave intensities during a pulsating ELF hiss event. Solid line, unfilled loss cone; dashed line, a loss cone partially filled in.

As the precise footprint of the GEOS 2 field line is uncertain and should move with the depression of the geomagnetic field during a substorm, more general attempts were made to relate the ELF to optical patches observed by all-sky cameras. Several studies were made to perform such a two-dimensional correlation using methods of multiple exposure photography but all proved unsuccessful⁷.

The size of most patches (<50 km across¹) is small compared with the uncertainty (>200 km) in the GEOS footprint location and small compared with the whole display of pulsating aurora which usually at some stage fills the whole sky as seen from any one point on the ground. Furthermore adjacent patches pulsate asynchronously with rapidly changing periods. The consequent difficulty in determining a precise footprint for the GEOS 2 field line relative to any one patch is probably the major factor hindering further progress in GEOS/ground correlated measurements of auroral pulsations.

The present study was attempted with the aim of verifying that the ELF hiss at GEOS is related to pitch angle scattering of energetic electrons at the equator. Although the precipitating electrons associated with pulsating aurora range in energy from keV to several tens of keV, this study concentrates on the higher energy electrons because of technical difficulties in observing fast-time variations at lower energies. Electrons with energies >22 keV are measured on the GEOS spacecraft with an electron spectrometer with directional sensitivity in 10 directions¹¹, arranged in steps of 10° to the spacecraft spin axis. The angular resolution is ±3° in angle away from the spacecraft spin axis and ±2° in spacecraft spin phase.

When the direction of the geomagnetic field is steady, one of these detectors will sweep through the narrow equatorial loss cone (~3°) once per spin of the spacecraft (5.9 s).

The data set used here is a 14-min period from 22.22 UT to 22.36 UT on 19 November 1979. This period was chosen because (1) the wave experiment¹² was configured to measure ELF wave amplitudes every 86 ms, (2) the geomagnetic field was constant in direction, (3) pulsating ELF hiss was occurring and (4) electrostatic VLF wave data were available with faster than usual time resolution as a result of manual telecommanding of the spacecraft. Also pulsating aurora was known to be occurring in Scandinavia at about this time (G. Gustafsson, personal communication).

For this data set the ELF (150–450 Hz) wave amplitudes observed in the 86-ms sample nearest to times at which the loss cone electrons were measured have been sorted according to whether the loss cone is partially filled or not. Partial filling of the loss cone was arbitrarily defined as being when the minimum electron count rate in the loss cone was >0.4 of that

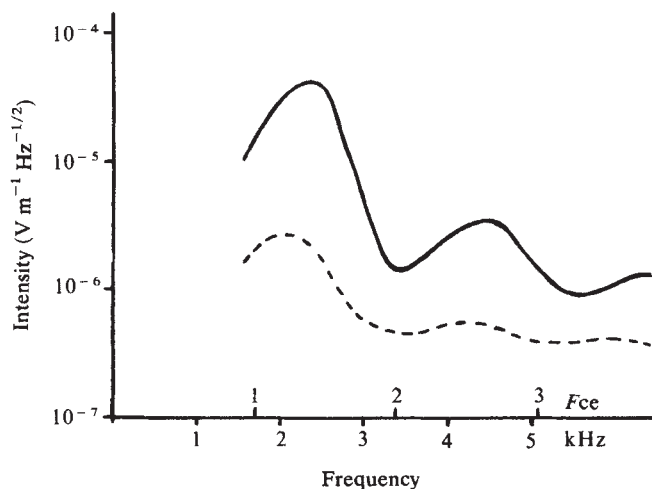


Fig. 2 Average electrostatic cyclotron harmonic wave spectra for this event. These spectra are the averages from the stepped filter analysers of the S 300 wave experiment stepping over 16 frequencies with a bandpass of 300 Hz. Solid line, unfilled loss cone; dashed line, a loss cone partially filled in.

count rate just outside of the loss cone. The opening angle of the detectors is roughly twice the angular width of the loss cone at the geostationary orbit and this ratio of count rates should therefore not be directly interpreted into particle fluxes. Each spin of the spacecraft will provide an apparent measurement of a smoothed out loss cone but the measured ratio will still increase with increased filling in of the loss cone.

Normalized histograms of occurrence of the different ELF hiss intensities are shown in Fig. 1 for the cases of unfilled loss cone (solid line) and partially filled loss cone (dashed line). When the loss cone is not filled in, the most likely ELF intensity is 10^{-5} nT Hz^{-1/2} with a 75% probability of the intensity being less than 10^{-4} nT Hz^{-1/2}. The lowest bin corresponding to 10^{-5} nT Hz^{-1/2} is occupied by 61 out of the 109 cases of unfilled loss cone but only by two cases out of the 23 cases of filled-in loss cone. On the other hand, when the loss cone is partially filled in, the most likely ELF intensity is two orders of magnitude higher around 10^{-3} nT Hz^{-1/2} with a 75% probability of the intensity being $>10^{-4}$ nT Hz^{-1/2}. Throughout this period the average count rate outside the loss cone remained constant and it is possible to calculate the error in estimating the ratio of count rates inside and outside the loss cone as ±0.07 for a ratio of 0.4. Such an error can be seen to be small by considering the spread of values occurring in this ratio. For example, all 63 cases of wave intensity $<2 \times 10^{-5}$ nT Hz^{-1/2} are spread evenly with ratios ranging from 0.2 to 0.4 with none above 0.45, whereas the 45 cases of wave intensity $>10^{-4}$ nT Hz^{-1/2} have their ratios spread evenly over twice the range, namely from 0.2 to 0.6.

Dividing the observations into (1) loss cone filled in (>0.4) or not and (2) ELF wave intensity $>10^{-4}$ nT Hz^{-1/2} or not yielded a $\chi^2 = 20.45$ for this one degree of freedom. Thus the relationship between ELF wave intensity and loss cone filling of >22 keV electrons is highly significant with a probability of observing this distribution by chance being $<10^{-4}$.

On auroral field lines ELF hiss will usually only be in cyclotron⁴ resonance with electrons ≥ 10 keV, but rocket measurements¹⁻³ showed that precipitation is observed down to ~2 keV. Electrons in this lower energy range are usually scattered in pitch angle by resonant interactions¹³ with the electrostatic cyclotron harmonic (ECH) waves found above the local gyro-frequency (f_{ce}). At the time of this data set the local plasma frequency f_{pe} was $\sim f_{ce}$ and the ECH waves observed were the class I and II in the classification scheme of Gough *et al.*¹⁴, that is, most of the emission is found in the first couple of gyro-harmonic bands.

Figure 2 shows the average ECH wave spectra for this data set when the loss cone was not filled (solid line) and for when the loss cone was partially filled (dashed line). The ECH waves are clearly weaker by an order of magnitude in intensity when the electron loss cone is partially filled in. These ECH waves obtain their free energy for growth from the gradients in phase space density on the edge of the loss cone¹⁵ and any filling in of the loss cones would tend to remove their source of free energy giving the observed reduction of wave amplitude. Therefore ECH waves are unlikely to be responsible for the increased scattering of the lower energy electrons required simultaneously with that of higher energy electrons by the ELF since any scattering produced would be in antiphase to that produced by the ELF.

We have shown that equatorial pulsating electromagnetic ELF hiss is directly related to the precipitation of electrons >22 keV during pulsating aurora. However, this result, rather than simply demonstrating the source mechanism, leaves us with more questions unanswered.

First, this evidence strongly favours a scenario where the ELF waves are pitch angle scattering electrons into the loss cone, but causality has not been established. It is even possible that another phenomenon is the driving force for both the ELF waves and the electron pitch angle scattering.

Second, what causes the ELF to be modulated with these pulsations? Coriniti and Kennel⁴ showed that micropulsations

could modulate ELF but as yet no simultaneous micropulsations have been observed in the geomagnetic field at geostationary orbit. However, Ward and Lester (personal communication) report a correlation between some events of pulsating ELF at GEOS and micropulsations measured on the ground.

Third, the relationship has been made with electrons >22 keV but auroral electrons range down in energy to ~ 2 keV. At these energies ELF waves are not likely to produce much pitch angle scattering so one needs to invoke another simultaneously occurring mechanism to account for low energy electrons during pulsations. Note that rocket measurements of pulsating electron precipitation^{2,3} show that both the low and high energy electrons all fit to the same spectrum, strongly indicating a single source mechanism.

Received 3 February; accepted 20 May 1982.

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We have, therefore, established a relationship between pulsating ELF hiss and precipitating electrons >22 keV at times of pulsating aurora. Future research must aim at defining the precise role played by the ELF waves.

We thank the staff at ESOC (Darmstadt) and CNES (Toulouse) for their efficient production of high quality data, also our colleagues in the GEOS collaboration, in particular Dr P. J. Christiansen, Dr A. D. Johnstone and Mr M. Townend for helpful discussions. Analysis of the particle data was supported by the Max-Planck-Gesellschaft zur Förderung der Wissenschaften and by the Ministerium für Forschung und Technologie through the DFVLR-BPT under contract no RV 14-B 12/73 (WRK 243)SF 21. Analysis of the wave data was supported by the SERC.

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Fission-track evidence for Quaternary uplift of the Nanga Parbat region, Pakistan

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The north-striking Nanga Parbat–Haramosh Massif protrudes into the northwestern Himalaya along the axis of a great syntaxis^{1,2} (Fig. 1), where the Hindu Kush, Karakorum, and Himalayan ranges converge. As the Indus Suture Zone³ enters this region from the east it bifurcates into two branches, encircling what may be a docked island-arc terrane⁴. The southern branch (the Main Mantle Thrust) crops out on both flanks of the Nanga Parbat massif, forming a tight structural loop⁵. This massif and the adjacent terrane contain some of the highest peaks in the Himalaya; Nanga Parbat and the Indus River (located just 20 km away) define the world's greatest continental relief (6,930 m). We report here the discovery of unexpectedly young sphene, zircon and apatite fission-track dates from the Nanga Parbat–Haramosh Massif. These dates (as low as 1.3 Myr for zircon and 0.4 Myr for apatite) imply that during the Pleistocene the Nanga Parbat region was uplifted and eroded at nearly 1 cm yr^{-1} .

The Nanga Parbat–Haramosh Massif is composed of migmatized, high-grade metasediments (Salkhala Series) of probable late Precambrian age^{1,2,5–7}. Metavolcanics, sediments and plutonics of the Kohistan island-arc sequence flank the massif on the east, north, and west⁵. Pelitic metasediments and felsic intrusives of the Asian mass predominate in the region immediately north of the arc sequence⁵.

Fission-track dates from all three of the above terranes (Table 1 and Fig. 2) reveal that in general, zircon fission-track dates become younger to the north (Figs 2, 3). Note that relatively young fission-track dates are associated with the trend of the Nanga Parbat structural loop and its northward extrapolation. In distinct contrast, older dates prevail away from the massif.

At elevated temperatures, fission tracks fade, and as a result, fission-track dates record the time when the dated material passed through its closure temperature⁸. These closure temperatures, which vary from mineral to mineral, are cooling-rate dependent⁹. At the cooling rates prevalent in the Nanga Parbat region (~ 10 to $>100^\circ\text{C Myr}^{-1}$; see Fig. 4 and below), we estimate the closure temperature for apatite to be $\sim 150^\circ\text{C}$ (refs 10–12), for zircon to be $\sim 235^\circ\text{C}$ (ref. 12) and for sphene to be $\sim 285^\circ\text{C}$ (ref. 12).

If cooling is the result of uplift and erosion, then a tectonic interpretation of fission-track data becomes possible^{13–16}, provided we know the local distribution of temperature with depth. From our previous work in the Pakistani Himalaya¹⁶, we have found 30°C km^{-1} to be a reasonable mean value. Since isotherms are deflected beneath topography¹⁷, especially in a region of extreme relief, a locally higher thermal gradient is likely in the Nanga Parbat area. For present purposes, we

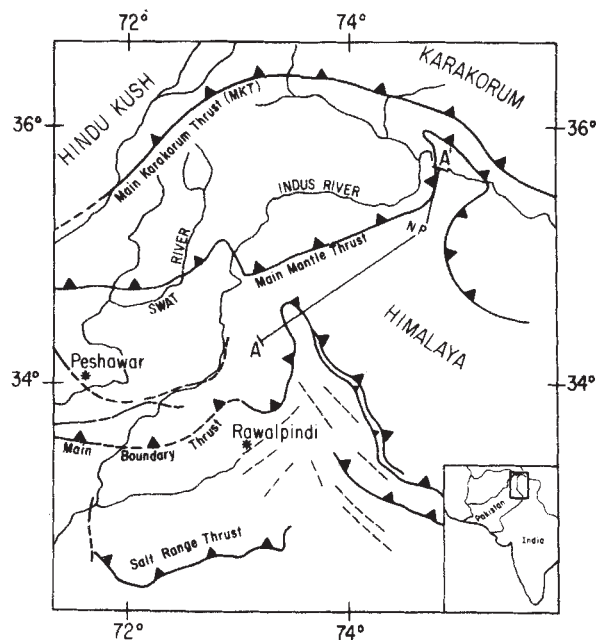


Fig. 1 Structural trends of northern Pakistan. Heavy barbed lines show suture zones and major thrust faults. Dashed lines show trends of folds in molasse sediments. NP, Nanga Parbat (8,126 m), located on Nanga Parbat–Haramosh Massif. A–A' is line of profile shown in Fig. 3.

Table 2 Sample elevations and uplift rates

Sample	Elevation (m)	Uplift rate (mm yr ⁻¹)*		
		Zircon-surface	Zircon-apatite	Apatite-surface
80Kg-1	2,970	0.37 ± 0.02 (7.6)	—	—
80Kg-2	3,190	0.44 ± 0.03 (6.4)	—	—
80I-6	4,440	4.3 ± 1.3 (0.7)	4.9 ± 5.4 (1.1)	4.0 ± 1.1 (0.4)
79I-4	1,480	3.3 ± 0.7 (0.9)	—	—
NPG	1,725	3.0 ± 0.6 (1.0)	1.4 ± 0.4 (1.1)	9.0 ± 2.8 (0.2)
79I-11	945	0.14 ± 0.01 (2.1)	—	—
80I-4	1,120	0.33 ± 0.03 (8.4)	—	—
79I-6	1,335	—	—	5.3 ± 1.6 (0.3)
LG, 79I-3	1,600	0.49 ± 0.03 (5.8)	0.24 ± 0.03 (7.1)	1.4 ± 0.4 (1.3)
79I-1	2,005	3.1 ± 0.4 (0.9)	2.0 ± 0.7 (1.3)	4.8 ± 1.6 (0.4)
AKG	2,390	1.8 ± 0.3 (1.6)	0.8 ± 0.1 (1.9)	6.9 ± 1.8 (0.3)

* Assumed geotherm is 40 °C km⁻¹. Closure temperatures: zircon, 235 °C; apatite, 150 °C; surface, 10 °C. Uncertainties reflect only uncertainties in ages. Values in parentheses are mean of age range over which uplift rate applies.

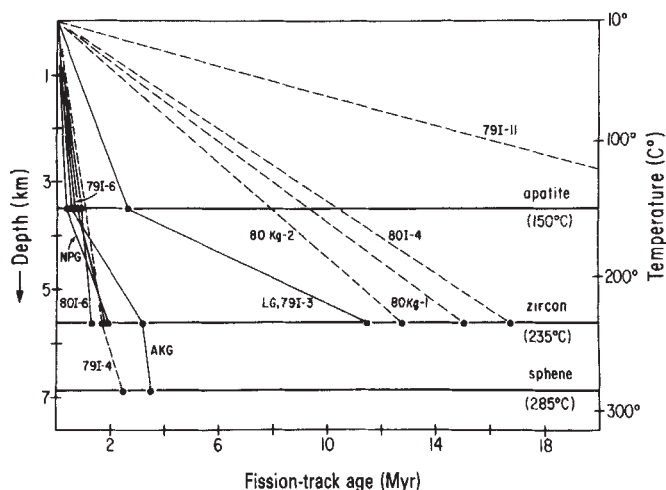


Fig. 4 Uplift/erosion and cooling trajectories of basement samples from the vicinity of the Nanga Parbat–Haramosh Massif. Uplift rates of nearly 10 mm yr⁻¹ apply to the massif (Table 2), and are represented by the steepest portions of the trajectories. Differential uplift is shown by the differing trajectories for samples 79I-6, NPG, 80I-6, 79I-4, AKG versus samples LG and 79I-3 (Fig. 2). Geothermal gradient used in construction of plot is 40 °C km⁻¹.

Estimates of uplift rates for the recent past and for the present do not exceed ~1 mm yr⁻¹, and most fall into the range 0.5–1 mm yr⁻¹, based on geodetic, geochronologic, metamorphic grid, and rate-of-denudation arguments.

Although our sample coverage is sparse, several general conclusions are evident. First, the high-uplift zone (defined by zircon dates <3 Myr and apatite dates <1 Myr (Fig. 2)) extends north past the Nanga Parbat–Haramosh Massif proper, past the 'nose' of the Main Mantle Thrust structural loop, across the northern branch of the Indus Suture (Main Karakorum Thrust), and onto the Asian landmass (Fig. 2). Second, uplift rates diminish rapidly away from the Nanga Parbat–Haramosh Massif. Samples LG and 79I-3, located on the island-arc terrane, are relatively close to the Nanga Parbat–Haramosh Massif. However, their pooled zircon age of ~11 Myr and apatite age of ~2.4 Myr are significantly greater than those of the nearby Nanga Parbat samples, suggesting that steep differential uplift has occurred. Third, note that the centre of maximum uplift, the Nanga Parbat–Haramosh Massif, is located at the focus of the northwestern Himalayan syntaxis (Fig. 1), perhaps not just by coincidence. Lastly, an inverse correlation exists between zircon fission-track age and peak elevation over a large geographical region (Fig. 3). This implies that in these mountains, elevation/relief is proportional to uplift rate.

This research was funded by NSF grants EAR 8007638 and INT 8019373. We thank John Lyons for helpful comments.

Received 22 February; accepted 5 May 1982.

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Speleothem dates, Quaternary terraces and uplift rates in New Zealand

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Previous isotopic research on calcite stalagmites, stalactites and flowstones (speleothems) from New Zealand caves has focused on palaeotemperature reconstruction^{1,2}. I report here the use of ²³⁰Th/²³⁴U and ¹⁴C age data from speleothems to investigate the relationships between cave levels, emerged coastal terraces and uplift rates. In the north-west of the South Island of New Zealand, at least seven terraces, provisionally assigned to various stages in the Quaternary, are known to occur to at least 200 m above sea level^{3,4}. The terraces sometimes cut across limestones which contain caves formed in association with past sea levels. Uplift, followed by water table lowering and abandonment of cave passages by active streams, permitted speleothem deposition. Dating of these deposits gives a minimum age for the cave level and hence also for any terrace to which it may be related, while successive dates at different levels yield an uplift rate for the area. Data are presented from two localities, which suggest differential emergence rates of 0.27^{+0.28}_{-0.09} mm yr⁻¹ and 0.14^{+0.03}_{-0.03} mm yr⁻¹. Thus re-evaluation is required of other largely qualitative evidence on the uplift of these parts of the Southern Alps⁵.

New Zealand is a tectonically active country. Rates of uplift are of considerable interest to those studying palaeosea levels⁷,

Table 1 $^{230}\text{Th}/^{234}\text{U}$ ages for New Zealand speleothems

District	Sample no.	Cave	Site	Height above base level (m)	Uranium concentration (p.p.m.)	$^{234}\text{U}/^{238}\text{U}$	$^{230}\text{Th}/^{234}\text{U}$	Age (kyr BP)
Takaka	2	Irvine's	Upper level	49 r	$0.040 \pm 0.003^*$	$0.98 \pm 0.08^*$	$0.99 \pm 0.08^*$	> 195
	3	Manson's	Lower level	210 r	0.056 ± 0.002	1.06 ± 0.03	2.12 ± 0.09	excess ^{230}Th , no age possible
Paturau	7	Wet Neck	Upper level	60 s	0.86 ± 0.05	1.49 ± 0.07	1.10 ± 0.04	450^{+55}_{-100}
	9b	Wet Neck	Upper level	60 s	0.75 ± 0.03	1.74 ± 0.08	0.73 ± 0.03	125 ± 10
	9c	Wet Neck	Upper level	60 s	—	2.57 ± 0.17	0.736 ± 0.036	122 ± 10
	9d	Wet Neck	Upper level	60 s	—	2.29 ± 0.09	0.686 ± 0.022	108 ± 5
	10	Penguin	Maze floor	4 s	0.28 ± 0.08	1.4 ± 0.4	0.056 ± 0.019	6 ± 2
	11	Penguin	Maze wall	5 s	0.40 ± 0.01	2.00 ± 0.07	0.395 ± 0.029	52 ± 6
	12	Cascade	Upper level	38 s	0.94 ± 0.05	1.36 ± 0.07	0.969 ± 0.061	275 ± 70
	13	Puponga	Ledge	5 s	0.27 ± 0.05	1.0 ± 0.2	0.182 ± 0.033	18 ± 4
	24	Metro	Derf section	27 r	0.08 ± 0.005	0.68 ± 0.04	0.67 ± 0.13	120 ± 45
	25	Metro	Biclops	30 r	0.02	1.5	0.3 ± 0.18	50 ± 30
	26	Metro	Sticky Trap	18 r	0.08 ± 0.01	0.98 ± 0.11	0.251 ± 0.04	31 ± 6
Westport	27	Metro	Hall of Refugees	11 r	0.05 ± 0.004	1.21 ± 0.09	0.338 ± 0.038	44 ± 5
	28	Metro	Conference Walk	14 r	0.77 ± 0.02	1.07 ± 0.03	0.367 ± 0.015	50 ± 3
	29	Metro	Paddy Fields	9 r	1.1 ± 0.02	1.12 ± 0.02	0.32 ± 0.010	42 ± 2 ($35.3^{+1.3}_{-1.0}$)
	30	Metro	Pigalle Corner	2 r	0.28 ± 0.04	1.06 ± 0.15	0.16 ± 0.03	18 ± 4 (16 ± 0.14)
	31b	Metro	Cave Pearls	2 r	1.2 ± 0.04	1.31 ± 0.04	0.190 ± 0.01	23 ± 3 ($28.9^{+1}_{-0.9}$)
	31c	Metro	Cave Pearls	2 r	1.1 ± 0.02	1.41 ± 0.03	0.223 ± 0.008	26 ± 1

Figures in parentheses after $^{230}\text{Th}/^{234}\text{U}$ ages are ^{14}C ages on same samples. s, Height above sea level; r, height above river level.

* Errors indicated are one standard deviation.

crustal deformation near the Indian-Pacific plate boundary⁸⁻¹¹ and the origin of the Southern Alps^{5,6}. The ages of uplifted marine terraces have often been central to the arguments but there are usually no dateable deposits on them. An exception is where volcanic ash overlies coastal terraces, and Yoshikawa *et al.*¹¹ have successfully dated this by fission-track techniques to yield minimum ages for the terraces concerned. Further opportunities arise for dating terraces where they cut across limestone lithologies with caves, because the latter often contain speleothems suitable for dating by ^{14}C or $^{230}\text{Th}/^{234}\text{U}$ disequilibrium methods.

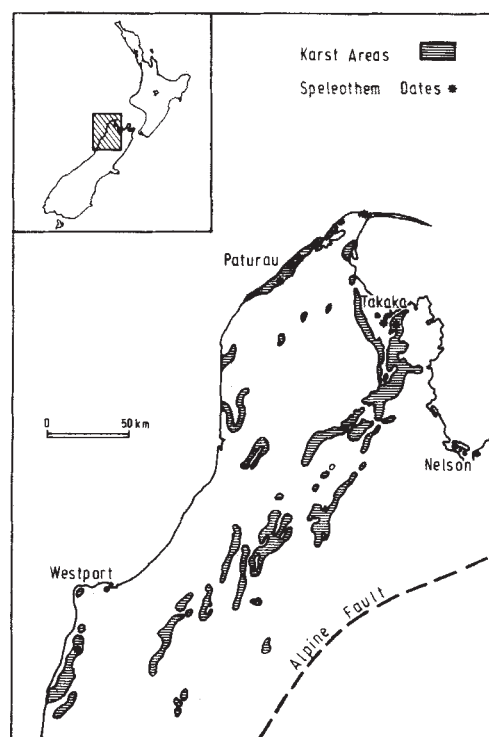


Fig. 1 The north-west of the South Island of New Zealand, showing areas where karstified limestones occur and sites where speleothems have been dated.

An area of particular interest is the northern part of the west coast of the South Island (Fig. 1). It lies to the north-west of the Alpine Fault, a major transcurrent dislocation with 500 km dextral displacement. An important series of coastal terraces has been described in the area^{3,4,12}, at least seven terraces having been recognized up to +200 m, but their ages are speculative. In the Westport area, Suggate³ has assigned a last interglacial (Oturian) age to two terraces between +34 and +45 m and an interglacial before last (Terangian) age to a +65 to +72 m terrace. A similar age has been suggested by Bishop¹² for the major +55 to +60 m terrace in the Paturau area of north-west Nelson further north (Fig. 1). Limestone caves run beneath or close to some of these coastal terraces and the record from them provides the only known means of dating the sequences and deriving their rates of emergence.

The karst of the Paturau area has been briefly described elsewhere¹³. An important characteristic of the region is that a 30–50 m zone of Tertiary limestones dips gently to the west-northwest, with the result that these rocks descend from +260 m at the summit of coastal hills in the north-east to below sea level in the south-west and, in so doing, obliquely cut across a set of emerged coastal terraces. Thus caves are associated with higher altitude terraces in the north-east than in the south-west.

The most well developed terrace at Paturau extends inland, usually for 400–600 m, to a height of ~+60 m at the foot of a degraded cliffline. A site of particular interest is Cascade Cave, an upper level phreatic passage of which occurs at +38 m and runs beneath the +60 m terrace at its inland end. The passage is essentially horizontal, meanders slightly and has a narrow, deep slot in its floor descending to a lower, active level. The passage has very little fill of any kind except for breakdown blocks and a few speleothems, one of which was sampled and dated by $^{230}\text{Th}/^{234}\text{U}$ methods as 275 ± 70 kyr old (Table 1). The implication, therefore, is that the +38 m passage is younger than the +60 m terrace, otherwise it would contain marine deposits, and that it dates from at least the interglacial before last.

This is not the case for Wet Neck Cave situated 3.6 km to the north-west. The upper part of this cave was in existence before the +60 m sea which invaded it, filling it to the level of the terrace (Fig. 2) with well rounded boulders, cobbles, and pebbles of igneous and metamorphic origin identical to those seen on the modern beach, but of foreign rock type to that found in the catchment of the cave. The fill also contains broken

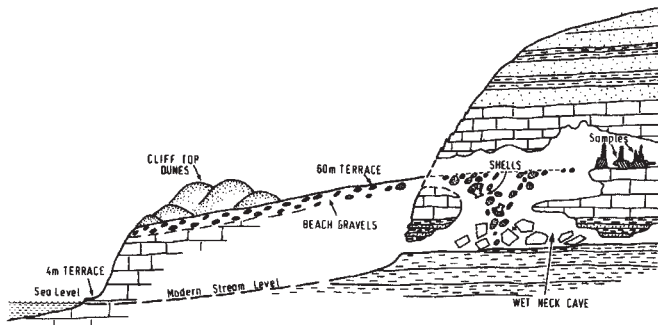


Fig. 2 Generalized section through Wet Neck Cave and the +60 m terrace at Paturau.

remnants of *Macra* sp. or *Dosinia* sp. shells; species which occupy open sandy subtidal waters at present. A stalagmite sample collected from a site close to and level with the top of the fill, but not actually on it (Fig. 2), was dated three times (Sample 9, Table 1), the replicate determinations yielding ages of 108 ± 5 , 122 ± 10 and 125 ± 10 kyr, indicating that the formation was growing in the last interglacial. However, this stalagmite partly overgrows a still older speleothem, dated at 450^{+100}_{-100} kyr (Table 1), that lies on the passage floor and may conceivably have been broken by the invading sea. Thus evidence from both caves indicates the +60 m terrace to be at least 275 ± 70 kyr old and possibly more than twice that age.

Much closer to sea level, a well developed but narrow rock platform often veneered with beach gravels occurs at about +4 m (Fig. 2). Just south of the Paturau River mouth, a fragmented phreatic maze cave (Penguin Cave) lies <300 m inland from the coast at $\sim +5$ m. It is not known whether the cave is directly connected to the terrace, because it is completely blocked with relatively fine sediments (probably of floodwater origin) at its seawards end, and no geomorphological evidence has been observed on the relative ages of the two features. However, speleothems in the cave, some of which are growing on the sedimentary fill, show no signs of having been affected by marine invasion since they were formed. Dating indicates this to have been up to 52 ± 6 kyr ago (Table 1). The neighbouring +4 m marine platform is thus likely to be of at least that age.

Near Westport (Fig. 1), 170 km further south along the same coast, a series of speleothem samples has been dated from passages at different levels in Metro Cave (Fig. 3), which is

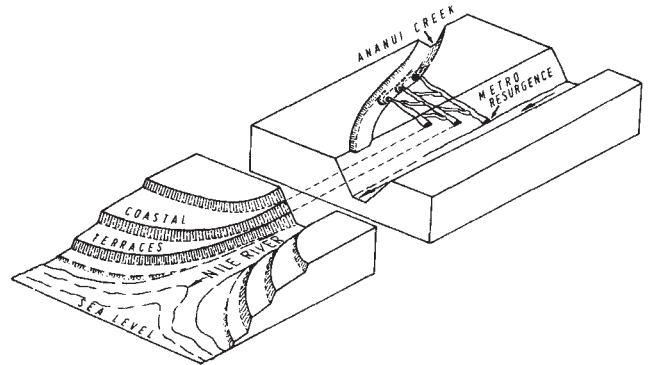


Fig. 3 Schematic block diagram showing the relationship of Metro Cave to emerged coastal terraces near Westport.

situated in a limestone gorge 5.5 km inland. The cave was formed by subterranean capture of Ananui Creek (Fig. 3) after incision of terrace gravels and a siltstone cap exposed underlying Tertiary limestones. The karstified drainage then followed a shorter and steeper underground route to the main river, joining it at a resurgence some distance upstream from its original surface confluence point. The resurgence level has since lowered as the trunk river has incised and has also moved upstream because of the inland dip of the limestone beds. The streamsink of Ananui Creek has also incised and migrated upstream.

The modern resurgence of the cave stream is at river level in the gorge at +30 m, but abandoned resurgences that may be assumed to have been once adjusted to previous higher river levels, which in turn were related to past sea levels, now occur in the gorge sides up to 37 m above the present river (Fig. 3). At no time since the formation of Metro Cave has any part of it been submerged by a transgressing sea.

Speleogenetic evidence shows the karstification of the Ananui Creek drainage to have occurred after the exposure of the terrace above the cave and no marine deposits are found in the cave. However, thick generally coarse fluvial sediments completely block some passages and occur as terraces in others, indicating phases of infilling and excavation that could be the result of variations in the load of Ananui Creek and of alluviation episodes at the resurgence.

Of the eight speleothems dated in Metro Cave, six were stalagmites situated on fluvial fill, one (sample 30) was seated

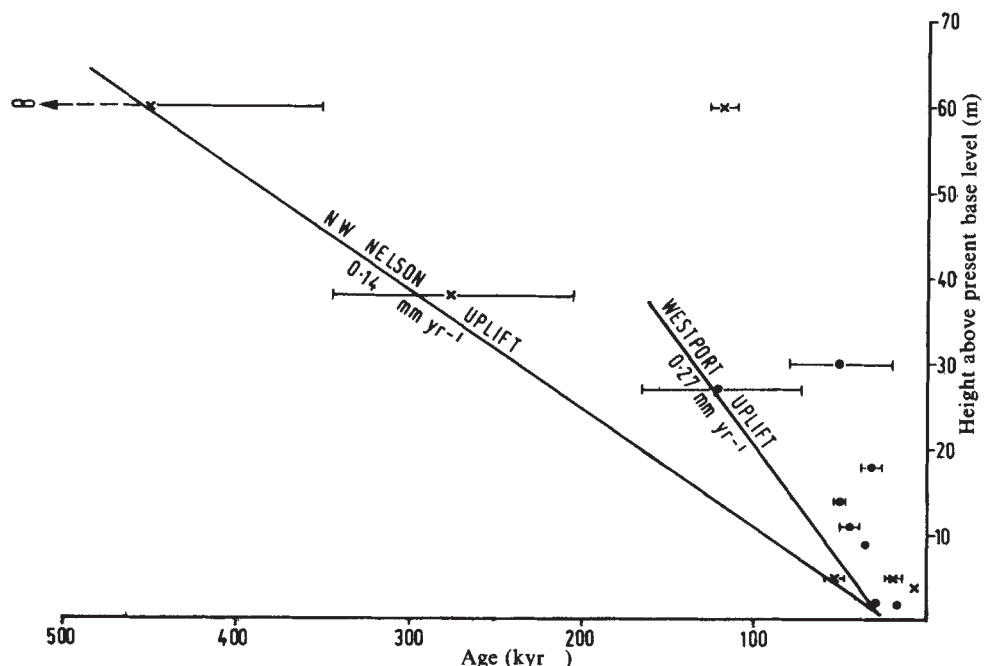


Fig. 4 Rates of uplift for localities near the north-west coast of the South Island of New Zealand. x, Paturau (NW Nelson) data; ●, Metro (Westport) data. Error bars derived from one standard deviation in counting statistics.

on bedrock in the present floodwater zone, and another (no. 31) was a stalactite. They were taken from widely spaced locations often at different levels and so cannot be readily stratigraphically related. Their age data (Table 1) show the cave to be at least 120 ± 45 kyr old, although the uppermost level could not be dated because of inadequate material for sampling. Note that samples dated by ^{14}C and uranium series methods (nos 29, 30 and 31, Table 1) are of similar order, although only one overlaps in age range.

If the Metro speleothem dates are plotted against height above the present local base level (the main river), an envelope line enclosing the upper age limit at each height defines the rate of downcutting of the river (Fig. 4), which may be assumed equivalent to the average rate of uplift for the Westport area. Age uncertainties derived from counting statistics result in a large error term on the rate derived, which is $0.27^{+0.28}_{-0.09} \text{ mm yr}^{-1}$. Nevertheless, given this uplift rate, the extensive well developed terrace above the cave (Fig. 3), which is an inland extension of the +65 to +72 m terrace at the coast⁴, can be estimated to be ~250 kyr old. This is compatible with the Terangian age suggested by Suggate³. Further, assuming the last interglacial sea level stood at +4 to +6 m some 125 kyr ago¹⁴, then the associated terrace in the Westport area would now be expected to be at about +39 m. Hence Suggate's³ assignment of the two terraces at +34 m to +36 m and +40 m to +45 m to the late and early phases of the last (Oturi) interglacial is also broadly supported by this evidence.

While the local base level at Metro Cave (the main river) has generally fallen, the base level affecting the caves at Paturau in north-west Nelson (sea level) has oscillated considerably through the Pleistocene and has consequently usually been different for caves of different ages. Nevertheless, using present sea level as a convenient reference datum, a plot of Paturau data against height shows the uplift rate from the envelope curve to be $0.14^{+0.05}_{-0.03} \text{ mm yr}^{-1}$ (Fig. 4).

Because this is only half the rate for Westport, differential uplift is inferred along the north-west coast of the South Island. However, because the margins of error on the uplift curves overlap, the rates could in fact be the same, although preliminary evidence on the relative heights of terraces in each area also supports tilting, although less than the uplift rates would imply. Initial results for the Takaka area of north-west Nelson (Fig. 1 and Table 1, samples 2 and 3) are inconclusive.

An interesting feature of both the Paturau and Westport localities is that speleothems appear to have been growing in at least two caves during the height of the last glaciation (sample nos 13 and 30, Table 1) about 15–18 kyr ago, although the error terms give some uncertainty. Nevertheless, this is contrary to much European and North American experience^{15–17}, which indicates speleothem growth to have diminished considerably during glacials, although there are exceptions¹⁸. It is therefore possible that on the west coast of the South Island of New Zealand during the last glaciation vegetation and glaciers were juxtaposed, just as they are in places at present. Active speleothems in Metro Cave in other relatively cold but not necessarily glacial periods support this proposition.

Uranium series dating of speleothems was conducted by C. H. Hendy whom I also thank for helpful comments on interpretation; shell fragments were identified by J. A. Grant-Mackie. The research was supported by grants from the Auckland University Research Fund.

Received 12 February; accepted 27 April 1982.

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Water chemistry control of cadmium content in Recent benthic foraminifera

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The micropalaeontology and isotopic chemistry of foraminifera and other fossils have provided a detailed picture of the surface ocean environment during glaciation^{1,2}. Less is known about climate-related changes in the deep ocean, although Weyl³ and Newell⁴ have proposed significant variations on theoretical grounds. It is clear that significant variations in the population assemblages and $\delta^{13}\text{C}$ of benthic foraminifera occur^{5,6} but these changes do not yet constrain a unique hydrographical solution. Adsorption of phosphate onto calcite and unfavourable solid solution behaviour^{7,8} preclude direct palaeonutrient determination. Because CdCO_3 and CaCO_3 form a continuous solid-solution series⁹ it has been suggested that the trace cadmium variability in foraminifera may provide an additional tool for probing changes in deep ocean circulation¹⁰. Here we demonstrate that certain benthic foraminifera, *Uvigerina* spp. and *C. kullenbergi*, show a consistent relationship between the Cd/Ca of the bottom water and of their calcite shells.

Figure 1 illustrates published covariance values^{11–15} between cadmium and phosphate at oceanographic stations throughout the world ocean. Because of the good correlation between the two elements (especially in deep water) a good estimate of cadmium in deep ocean waters can be derived from the phosphate concentration. This relationship has been shown to apply down to the sediment surface¹⁶. The covariance is useful in that considerable data are available on the phosphate distribution

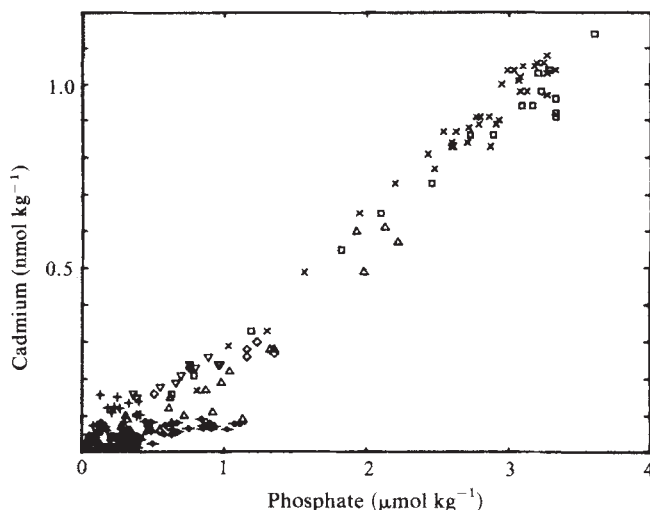


Fig. 1 Paired cadmium and phosphate data from ocean water samples reported recently (at depth, except where noted). Δ , northeastern Coastal Pacific from ref. 8; $\square$, northeastern Coastal Pacific from ref. 9; $\times$, northeastern Coastal Pacific from ref. 10; $\blacklozenge$, eastern equatorial Pacific surface waters from ref. 11; $+$, North Pacific surface waters from ref. 11; ∇ , Arctic Ocean from ref. 12.

Table 1 Benthic foram Cd/Ca in deep-sea core tops

Core	Location	Water depth (m)	Variety	Cd/Ca ($\mu\text{mol per mol}$)	Estimated bottom water PO_4 ($\mu\text{mol per kg}$)
AII107	Core 107GGC*	30°53' S, 37°48' W	2,795	<i>Uvigerina</i> spp. 0.095 (range ± 0.007 , $n = 2$)	1.4
				<i>C. kullenbergi</i> 0.086 (range ± 0.015 , $n = 2$)	
CH100	Core 87PG*	12°50.7' S, 168°38.1' E	3,473	<i>C. kullenbergi</i> 0.151 ($n = 1$)	2.4
AII107	Core 104GC*	31°16' S, 35°55' W	1,430	<i>Uvigerina</i> spp. 0.085 ($n = 1$)	2.2
AII107	Core 70GGC*	31°36' S, 35°59' W	2,079	<i>Uvigerina</i> spp. 0.091 ($\sigma = 0.010$, $n = 7$)	1.6
AII107	Core 73GGC*	31°25' S, 35°53' W	1,660	<i>Uvigerina</i> spp. 0.107 ($\sigma = 0.017$, $n = 5$)	1.9
				<i>C. kullenbergi</i> 0.098 ($n = 1$)	
AII54	Core 5PG†	7°25' S, 89°10' W	4,165	<i>Uvigerina</i> spp. 0.192 ($\sigma = 0.009$, $n = 3$)	2.6
				<i>C. kullenbergi</i> 0.170 ($n = 1$)	
AII54	Core 1PG	4°53' N, 83°26' W	3,395	<i>Uvigerina</i> spp. 0.163 ($n = 1$)	2.8
CH61	Leg-2 Sta 19				
	Core 1P	36°47' N, 13°6' E	833	<i>Uvigerina</i> spp. 0.042 ($n = 1$)	0.3
CH82	Sta 31				
	Core 11PC‡	42° N, 32° W	3,209	<i>C. kullenbergi</i> 0.059 ($n = 1$)	1.2
CH82	Sta 31 Core 11PG	42° N, 32° W	3,209	<i>C. kullenbergi</i> 0.054 ($n = 1$)	1.2
V29-184§		49°08' N, 25°30' W	3,645	<i>C. kullenbergi</i> 0.126 ($n = 1$)	1.3

* Recent status ascertained by D. Johnson and G. Jones (personal communication).

† Inferred Recent from $\delta^{18}\text{O}$ in core top²¹.

‡ Recent status confirmed by $\delta^{18}\text{O}$ profile (L. Keigwin, personal communication).

§ Recent status suggested by micropalaeontologic work (CLIMAP⁴).

in the deep ocean while information on cadmium is rather sparse, and direct culturing experiments on deep-sea benthic foraminifera are not feasible. Therefore we have selected a suite of deep-sea sediment cores where the deep water phosphate values can be estimated from published data^{17,18} (Table 1). Nine out of 11 of these core tops have been determined to be Recent by stratigraphical studies.

Specially cleaned handpicked specimens were dissolved in dilute nitric acid for analysis. Cadmium concentrations were quantified by graphite furnace atomic absorption spectroscopy as described previously¹⁰. Foraminifera are cleaned with a reducing/complexing reagent (hydrazine/citrate) to remove Fe/Mn oxide coatings and adsorbed ions. Typical samples were about 1 mg (15–40 individuals). The cadmium detection limit was $<10^{-12}$ g and the instrumental signal-to-noise ratio for samples was always >10 . Based on the standard deviation of replicates (Table 1) we estimate our precision on the Cd/Ca ratio as $\pm 0.010 \mu\text{mol Cd/mol Ca}$. At the low concentration

levels observed, contamination is always a possibility so samples represented by a single analysis should be given less weight than those confirmed by replicates.

The cadmium concentrations in the two species of core-top benthic foraminifera are plotted against bottom water phosphate concentrations in Fig. 2. The number of replicates per sample is represented by the symbol size. The cadmium content of the foraminifera tests increases linearly with increasing concentrations in the bottom water. Scatter in the plots only slightly exceeds our analytical precision and our ability to estimate bottom water phosphate concentrations by extrapolation from published sections (about $\pm 0.2 \mu\text{mol kg}^{-1}$). Using the Cd–P relationship illustrated in Fig. 1 we can use the data in Fig. 2 to compute an effective distribution coefficient $[(\text{Cd/Ca})_{\text{foram}} = D(\text{Cd/Ca})_{\text{seawater}}]$ $D = 2.0 \pm 0.4$ for the fractionation of cadmium in the calcite of these benthic foraminifera relative to the water in which they grew. This estimate is valid only for cold deep waters ($\sim 3^\circ\text{C}$). Such a relationship is consistent with solid-solution thermodynamics, although there are no data for comparison at the relevant temperature. There is no significant difference between the two varieties, as observed both by the trends in Fig. 2 and in core tops where the two varieties coexist. Other types of benthic foraminifera were also analysed but did not show as good a correspondence as these. Similar differences between varieties of benthic foraminifera have been reported in attempts to calibrate $\delta^{13}\text{C}$ in benthic foraminifera^{19–21}.

We conclude that the shell composition of these benthic foraminifera responds to the chemical composition of the water they grow in. This relationship can be utilized to reconstruct chemical variations of the ocean from the fossils in deep-sea sediment cores. Recent work on the variability of Cd/Ca in benthic foraminifera from the North Atlantic (core CHN82 Sta 31 Core 11PC) demonstrate that systematic climate-correlated changes in ocean chemistry occur over the past 150,000 yr (ref. 22): Cd/Ca increases from 0.059 in the recent core top to $0.10 \mu\text{mol Cd/mol Ca}$ at the glacial maximum. Data on the chemical variability of past oceans will give us an insight into changes in deep ocean circulation with time.

We thank David Johnson for access to sediment cores, and Jim Broda for help with other sampling, also M. Delaney, M. Bender, D. Graham, L. Keigwin and W. Curry for helpful discussions. Core collection and curation at the Woods Hole Oceanographic Institution is supported by NSF grant OCE 20/25231 and that at the Lamont-Doherty Geological Observatory by NSF grant OCE 7825448. This research was sponsored by NSF grant OCE-8018665.

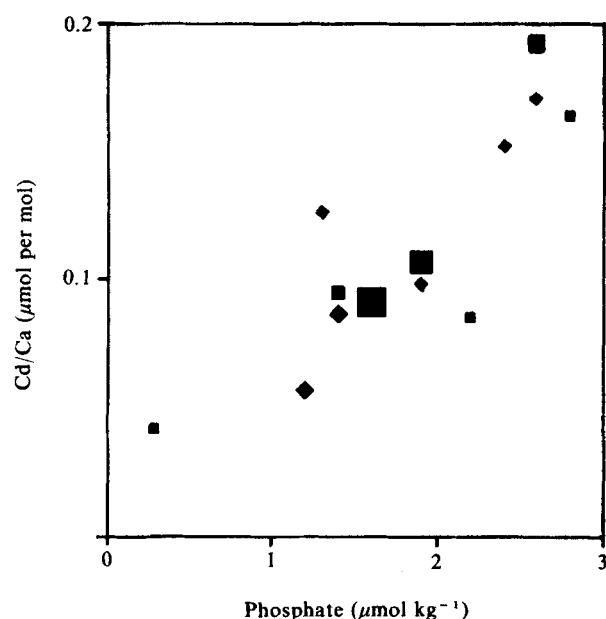


Fig. 2 Cd/Ca in recent benthic foraminifera plotted against estimated bottom phosphate concentrations. The size of the symbol increases with increasing numbers of replicates (Table 1).

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In situ metal-staining of biological membranes in sediments

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Decomposition of organic matter in sediments is principally mediated by bacteria and burrowing organisms. During this process, organic matter undergoes a series of structural changes in which heavy metals and minerals actively participate^{1–4}. Metal-organic complexes arise which are instrumental in crystal seed formation and growth of authigenic minerals such as sulphides, phosphates, oxides and carbonates. By means of transmission electron microscopy, modern organic-rich sediments from the Black Sea and Lake Tanganyika and samples from Eocene Messel oil shale in Germany were studied for the presence of heavy metal stained biological membranes. We report here that during early diagenesis heavy metals seem to supplant hydrogen, alkali and alkaline-earth ions placed at the surfaces of membranes in living organisms. The resulting patterns are the same as those found in organic tissues which before electron microscopy were stained with lead, uranium and osmium salts. In sediments, naturally stained membranes can further act as templates for mineral deposition and may—in certain environmental conditions—lead to strata-bound ore deposits.

Metal ions are a key factor in the structural organization of many biochemical molecules⁵. They form coordination complexes with oxygen, nitrogen and sulphur donor groups present in such molecules and introduce a higher structural order. A model on the role of metals in biological systems has been presented recently⁶. It emphasizes the similarity of structural patterns observed in biological and geochemical systems and shows that metal-oxygen polyhedra are the basic structural building blocks in both these systems. Recent studies further indicate that metal deposition in microorganisms is also promoted by metal-organic interactions^{7,8}. The major role of organic matter appears to be related to the supply of oxygen functions (for example, carboxyl, hydroxyl, carbonyl groups) for the generation of metal-oxygen polyhedra. This can be the starting point for the oriented growth of minerals.

During early diagenesis organic matter undergoes a series of alterations, which are mainly microbially and abiotically mediated. One of the processes involves the natural staining of

organic matter by metals⁹. This is a result of formation of coordination bonds between metals and oxygen donor groups present in the organic matter¹⁰. Metal-oxygen polyhedra subsequently act as templates in the nucleation and epitaxial growth of distinct mineral phases. Depending on the type of substrate, prevailing environmental conditions and microbial system, formation of oxides, phosphates, carbonates and sulphides may proceed. Structures resembling the reality of these mechanisms have been observed previously^{9,11}. In fact, preservation of organic matter and organic structures, especially the soft tissues of organisms through geological time, is a consequence of metal staining.

The palaeobiological significance of such processes was first demonstrated in the 1930s during investigations on geological samples of the Eocene brown coal of Geiseltal in Germany¹². Microscopic investigations of these samples revealed well-preserved histological structures. The excellent preservation was suggested to be partly due to the formation of humic substances. In another study, thin slabs of Devonian shales, when subjected to X-ray analyses, contoured fossilized remains of organisms¹³ in great detail. The contrast was provided by tiny sulphide crystals covering these organisms immediately following their death and deposition.

We have extended these investigations using high resolution electron microscopy. Well preserved organic tissues in various stages of metal-fixation or mineralization were observed, revealing mechanisms of heavy metal fixation at the molecular level.

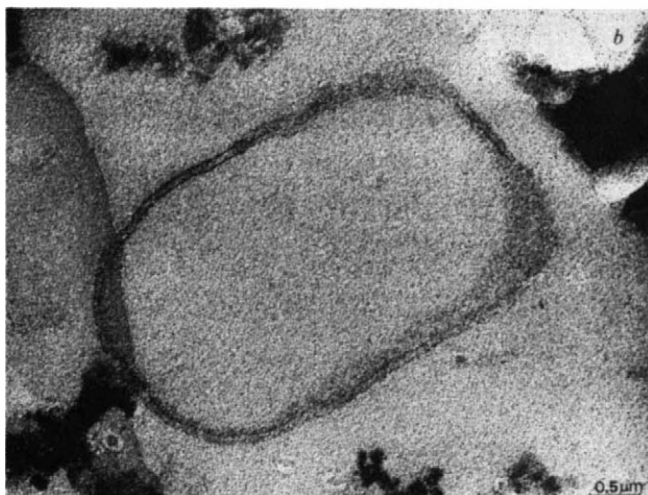
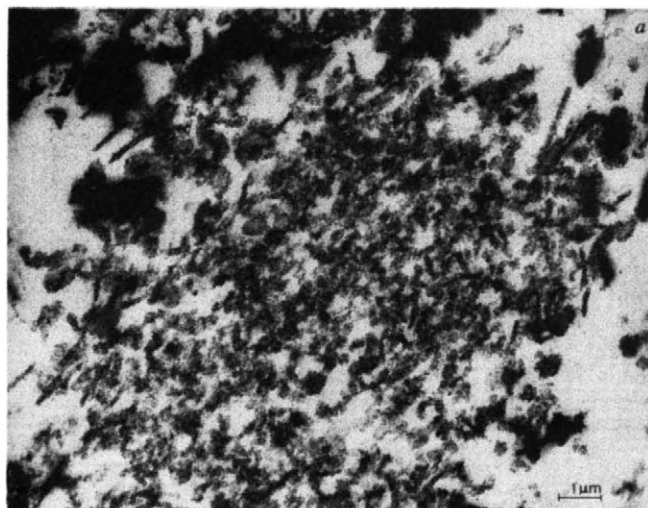


Fig. 1 Electron micrographs in Lake Tanganyika sediments of *a*, metal stained membrane fragments; *b*, bacterial membrane. The membrane bilayer is in various stages of disaggregation probably due to different degrees of metal fixation.

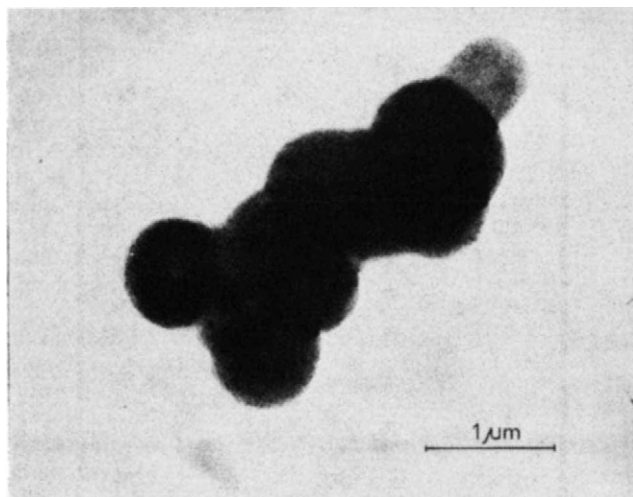


Fig. 2 Clumps of structures resembling bacteria (methanogenic?) and having a diameter of 1–2 μm . Membranes are visible through natural metal staining. Messel oil shale.

Electron microscopic investigations were carried out on sediment samples rich in organic matter from the Black Sea and Lake Tanganyika and on samples from the Eocene Messel oil shale in Germany. Samples from the Black Sea and Lake Tanganyika were studied after preparing ultrathin sections and those from the Messel oil shales directly. We now present results obtained after examining the bituminous samples without prior heavy metal staining.

A distinct feature of electron micrographs obtained from the study is the massive occurrence of membranes (Fig. 1) and of intact structures resembling bacteria (Fig. 2). The membranes are quite prominent in sediment and occur in different stages of aggregation. An interesting feature in Messel oil shales is the presence of clumps of spherical structures, that is, individual structures 1–2 μm in diameter. These structures are reminiscent of assemblages of methanogenic bacteria¹⁴. Molecular entities of membranes of methanogenic bacteria have recently been identified in the Messel oil shale^{15,16}. Significant among other structures observed during the study is the occurrence of organic tissues with tiny sulphide crystals embedded in them. In several cases, structures resembling bacteria are surrounded by idiomorphic sulphide crystals (Fig. 3).

Because bacteria are terminal members of the food chains it is not surprising that morphologically intact bacteria or their membranes are common in these sediments even to the extent of sediment layers a few micrometres thick. However, the significance of the structures does not rest solely in the resolution of these structures under the transmission electron microscope (TEM) but that these biogenic structures are visible without applying heavy metal staining before analyses. In TEM studies of biological specimens it is usual to examine them after staining with salts of certain heavy metals. The application of salt is needed to obtain high contrast images. More recently a modification of this technique has been used to study carbohydrates in soil organic matter¹⁷. Most of the organic remains in sediments that we studied were of high contrast images. We emphasize that high contrast was obtained without heavy metal staining, which suggests that it is solely due to natural (*in situ*) heavy metal staining. No difference in electron density was observed between specimens stained with lead and others that remained unstained. We conclude this to be due to an exchange of hydrogen or alkali and alkaline-earth ions fixed at the membrane surface for certain heavy metals present in interstitial solutions. Furthermore, metal ions coordinate to oxygen functions present in the organic matter and cause the formation of distinct metal–oxygen polyhedra^{6,10}. Such interactions may lead to coordination changes in the organic matter with resultant changes in molecular structure and shape.

The relevance of the study for palaeobiological investigations is apparent. Metal staining will render organic matter inert to biological degradation, increase its chemical and thermal stability and permit identification under TEM. It is significant that metal-staining will introduce coordination changes. We believe that organic tissues stained with a heavy metal, both naturally as seen in our study or artificially in biological investigations, exhibit a different structural state than the organic matter in its natural unstained form.

Metal–organic interactions and the formation of metal–oxygen polyhedra can further initiate metal deposition within or around the cellular material. The kind of organic structures generated will depend on (1) original source material, (2) environmental conditions in terms of *Eh*, pH, salinity and (3) type and availability of metal ions and minerals. Therefore interpretations based solely on such 'molecular artefacts' should be made with great caution. This is particularly true in biological research. For example, different staining techniques on the same sample will yield different structural patterns¹⁸. Furthermore, the use of a single staining material—phosphotungstic acid (PTA)—produced different structural patterns from the same starting material¹⁹. Penetration of PTA into biological specimens is pH dependent, and the same specimen studied under different pH was found to change its apparent structure. The effect of preparation of samples for biological studies has been discussed in great detail elsewhere^{20,21} and it has been suggested that many of the structures observed under the electron microscope are artefacts of preparation. It appears that, especially in studies aimed at deciphering membrane structures, new techniques involving no prior treatment of samples are urgently needed. One such method involves the emplacement of cell membranes or other tissues in heat and radiant-resistant oil (Corning Fluid 550) and subsequent electron diffraction^{6,22}. This method has the advantage that exposure times to the electron beam can be extended to a few minutes without changing the cellular material.

In conclusion, the widespread occurrence of metal-stained organic matter in the samples investigated indicates that enrichment of certain heavy metals in bituminous sediments are a result of such processes. However, organo-metallic interactions of this type appear to be an intermediary in the formation of various mineral phases, such as sulphides, as seen from the presence of organic matter in various stages of mineralization. We suggest that the role of organic matter in the depositional environment is similar to that observed in metal deposition in microorganisms. The role of organic matter is to provide functional groups for the coordination of heavy metals which, depending on the prevailing environmental conditions and



Fig. 3 Structures having diameters of 0.5–1.5 μm and surrounded by euhedral sulphide crystals. Black Sea sediments.

microbial populations, can facilitate the nucleation and growth of distinct mineral phases. Cell membranes due to their unique molecular composition, exhibit an especially high affinity for the fixation of heavy metals. Scavenging of metals from the environment by organics and subsequent mineral growth at the sediment-water interface is considered to be the prime factor in the generation of strata-bound ore deposits.

We thank Dr G. Müller for the use of and his help in carrying out electron microscopic investigations of the Messel shale samples at the Tropen Institut, Hamburg, FRG and the Shell Grants Committee, London for financial support.

Received 22 February; accepted 10 May 1982.

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Spatial organization of the white-fronted bee-eater

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White-fronted bee-eaters (*Merops bullockoides*, order Coraciiformes) have one of the most complex societies recorded among birds. These birds are highly gregarious, roosting and breeding in large colonies of up to several hundred individuals. They are also cooperative breeders, with over one-half of all breeding pairs being assisted by one or more helpers at the nest¹. Each colony is subdivisible into several smaller, tightly interacting social units, each consisting of several pairs and helpers—these units we term clans. Here we report that each bee-eater clan defends a group foraging territory that may be located as many as 7 km from the colony site. Each group territory is subdivided into several partially overlapping home ranges belonging to different members of the clan. These home range clusters remain spatially fixed despite periodic shifts from one colonial roosting and breeding site to another. We suggest that the spatial segregation of feeding territories and breeding colonies may have contributed to the complexity of the societal structure of these birds.

In January 1977 we began a continuous study of white-fronted bee-eaters in Lake Nakuru National Park, Kenya. The birds lived in colonies, consisting of 40–450 birds, which were located in vertical sand cliffs along the banks of the Makalia River, in the southern portion of the park. The bee-eaters excavated chambers in these cliffs which were used for both

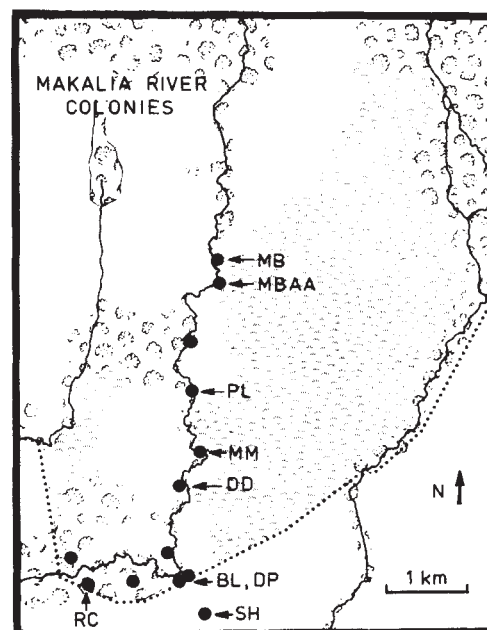


Fig. 1 Map of the study area in Lake Nakuru National Park, showing colony sites (solid circles) along the Makalia River. Labeled sites designate colonies at which breeding occurred during 1977–79. Vegetation types include *Acacia* woodland (stippled), wooded savannah (dotted) and grassland. The dotted line indicates the southern boundary of Lake Nakuru National Park.

roosting and nesting. All breeding occurred in the colonies, where all members of the resident population congregated each night to roost. The colonies thus served as social foci for the population throughout the year.

In the first 31 months of the study, we made observations of ~200 individually marked birds at colonies and on foraging territories along a 6-km portion of the Makalia River (see Fig. 1). We made detailed maps of four selected portions of the study area and, by plotting the locations of individually known birds onto these maps, we were able to quantify territory sizes and study the spatial arrangements of different clan members at the foraging areas.

Territories were located on either side, and generally within 1 km, of the Makalia River. Individual bee-eaters spent most of the day (during the non-breeding seasons) on their foraging territories and returned to the colony in the late afternoon. This necessitated daily round-trip commuting over distances of as much as 14 km.

When the social interactions of individually marked birds were followed through time, it became apparent that definite social subdivisions occurred within the larger context of the colony. Each bee-eater was a member of a smaller social unit consisting of 2–11 birds (median = 7), called a clan. Membership in a clan was defined operationally on the basis of the types and frequencies of interactions that occurred between individuals. Members of the same clan interacted repeatedly, on a daily basis, throughout their lives. At the colony they occupied specific chambers which they defended aggressively against outsiders. They greeted one another profusely during pre-roost gatherings, and non-breeding individuals generally acted as helpers, but only at the nests of other members of the same clan; such helping almost never occurred between members of different clans (S.T.E., unpublished data).

A clan typically consisted of one to five monogamous pairs of birds, plus a smaller assortment of single (unpaired) individuals. The latter were either young birds that had not yet paired (generally in their first year of life), or older, widowed individuals that had not yet re-mated. Birds became members of a clan in one of two ways: they were either born into the clan (remaining with their natal unit) or joined by pairing with

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a current member. This resulted in a clan kin-structure that was based largely on extended family relationships (S.T.E., unpublished data). Pairing was exogamous, and no cases of incestuous matings have been recorded (S.T.E., unpublished data).

Members of a bee-eater clan shared access to a common foraging area, from which they aggressively excluded all non-clan members. Eight intensively studied clans, comprising three to eight members (median=6), defended areas ranging in size from 7.4 to 18.7 h (median=8.8). The size of each area was positively related to clan size (Spearman's rank correlation coefficient, $r = 0.88$, $P < 0.01$). The rates of aggressive interactions between clans of *M. bullockoides* were low (45 cases in 140 h of observation, or a rate of 0.3 interactions per hour) compared with those of other territorial species, but boundaries between adjacent clans were sharply defined, mutually respected and relatively stable. Only one major boundary change occurred between the eight clans studied during the 31 months.

In contrast to many other group-territorial bird species which travel and forage in coordinated flocks within their territories²⁻⁷, members of bee-eater clans were dispersed in their foraging

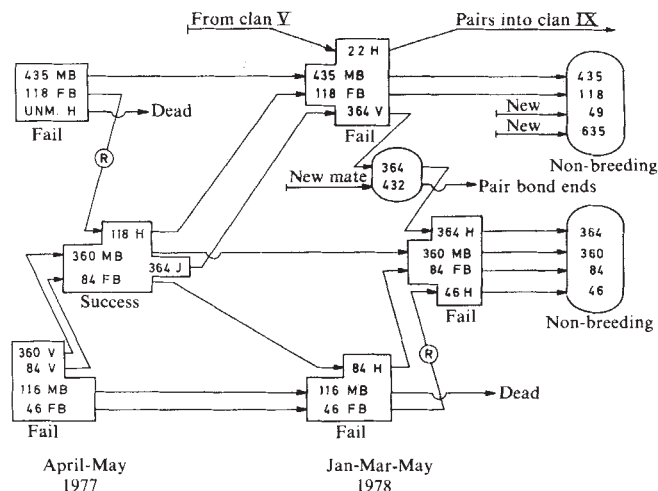


Fig. 3 Flow diagram for clan I. Individual birds are identified by their band numbers, and connecting lines trace their social movements with time. Clan I originally consisted of three monogamous pairs (435-118, 360-84 and 116-46), and one juvenile (364) fledged in 1977. In 1978 the juvenile paired briefly with another yearling (bird 432), but their pair bond dissolved shortly afterwards. Each box represents a breeding or roosting chamber in the colony. The birds residing in that chamber, and their sex and status, are shown in the boxes: MB denotes male breeder; FB, female breeder; H, helper, V, visitor (a bird that roosted in the chamber but did not help in the nesting effort); and J, juvenile. The outcome of each breeding effort is indicated below the box. Note (1) the fluidity of subunit associations in the clan; (2) the occurrence of redirected helping by some breeders whose nesting attempts fail (designated R); (3) the reciprocal exchange of helping between females 84 and 46; and (4) the restriction of all but one case of helping to within-clan members.

areas and showed no tendency to flock or feed gregariously (R.E.H., unpublished data). Instead, they divided the foraging area into a number of loosely overlapping home ranges, each used by a different clan subunit. Generally, each monogamously bonded pair used a distinct area, and hence the number of home ranges within the clan's group territory usually corresponded to the number of breeding pairs in the clan. The home ranges were not entirely exclusive, however, as the areas used by adjacent pairs of the same clan showed considerable spatial overlap (mean=39%, $n=7$ pairs). Aggressive behaviour between such pairs was rare, and typically consisted of only low-intensity threats. In 140 h of focal-individual sampling, we observed only 56 cases of intra-clan aggression (0.4 per hour); 31 of these cases were seen completely enough to classify them accurately. Only nine (29%) involved established pairs within a clan, and most of these were associated with mate guarding, which occurs during the week before egg-laying (S.T.E., unpublished data). Most of the remaining cases involved aggression directed at a new mate that was in the process of joining the clan.

The patterns of territory use by unpaired members of the clan were more complex. Young, unpaired clan members did not confine their movements to the home range occupied by their parents, but instead moved freely throughout the entire clan neighbourhood. On average, birds in their first 6 months of life overlapped as much with other pairs in the clan (35%, $n=6$) as with their parents (52%, $n=9$; Mann-Whitney test, $U = 16$, $P = 0.21$), although this measure was highly variable. When young individuals acquired a mate, they restricted their activities and established a home range of their own within the clan's group territory ($n=3$ cases).

Some widowed clan members remained unpaired for considerable periods of time. When this occurred ($n=3$), they began to associate closely with one of the other pairs in the clan, roosting in their chamber in the colony at night and helping them during the breeding season. Simultaneously, these widowed individuals began to associate closely with the same

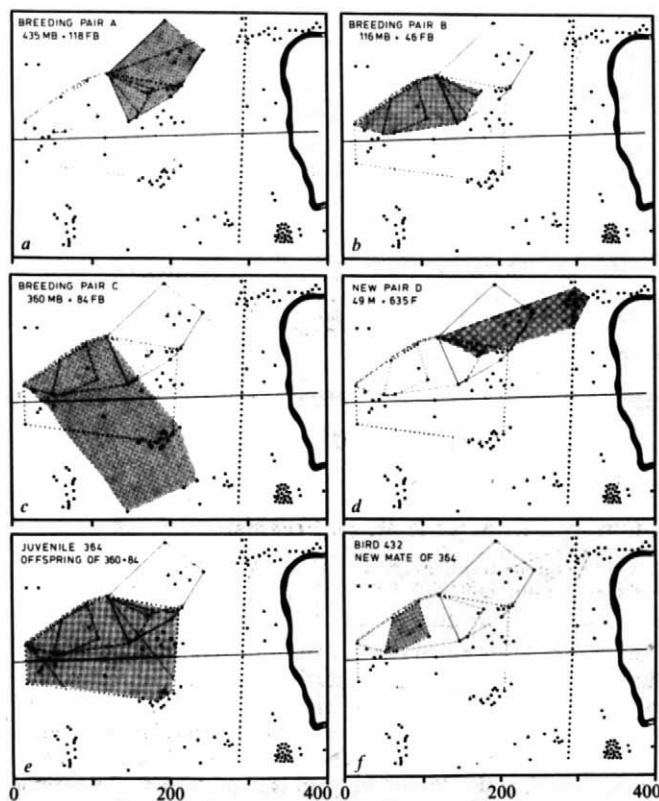


Fig. 2 Maps (1:300) of the territory neighbourhood controlled by clan I of the Makalia population. Shown in all six maps are the Makalia River (thick solid line), a telephone line passing through the area (narrow solid line), a line of fence posts (vertical line of open circles), all trees or other foraging perches (solid circles) and the outlines of the portions of the areas used by each monogamous pair or unpaired member of the clan. In each map, one of these areas is shaded for easier reference. Shown here are the home ranges of pairs 435-118 (a), 116-46 (b), 360-84 (c), 49-635 (d), bird 364 (e) and bird 432 (f). Note (1) the spatial overlap between adjacent monogamous pairs; (2) the overlap between bird 364 and monogamous pairs other than his parents (who were pair 360-84); and (3) the restriction of helping by bird 432 (a temporary mate of bird 364) to an area entirely within the area used by bird 364. This latter point illustrates an example of a new bird (432) joining an existing clan at their foraging area after pairing with a clan member (bird 364), and these two birds then beginning to establish a home range of their own within the clan foraging territory.

pair on the foraging area, shifting their activities away from their former home range and eventually overlapping spatially with the new pair almost entirely (eventual mean overlap = 89%).

The overall pattern of territory occupation by a bee-eater clan is best described as a neighbourhood of overlapping individual or pair home ranges. This type of spatial organization is rare among birds and is illustrated for a representative clan in Fig. 2. The social structure and behavioural interactions of this clan during the mapping period are shown in Fig. 3, and should be consulted when interpreting Fig. 2.

White-fronted bee-eaters typically change colony locations between successive breeding seasons. We have monitored 14 colonies over successive breeding years, and in 11 cases the birds moved to alternative colony sites. In the remaining three cases, the birds used a different set of nesting chambers at the same physical location. In the case of the Makalia population, there were seven colony sites along the 6-km stretch of river (Fig. 1); each had been used in the past, as evidenced by the large numbers of previously dug roosting chambers. Early in 1977, clan I (and most of the Makalia population) bred at MBAA colony in the northern part of the study area. In May 1977, this colony was flooded and the birds moved and re-nested in the nearby MB colony. Following a successful nesting, they continued to roost at MB colony until late 1977, when the entire population moved to MM colony, 2.4 km to the south. They bred at MM colony in 1978 and roosted there until early in 1979. They then shifted back to MBAA colony where they bred in April 1979. Following this they moved 2.6 km south to the DD colony.

Although the location of the roosting and nesting colony shifted with time, the location of each clan neighbourhood remained constant, and individual birds continued to use the same foraging area each day. For example, the defended area of clan I was located near MBAA colony. In some years (1977 and 1979), the breeding colony was located close to the clan's foraging territories, but in other years (1978) the colony was much farther away. Hence, over a period of years, a given clan's neighbourhood of territories will vary in its distance from the nesting colonies.

As far as we know, white-fronted bee-eater society is unique in combining territorial defense, colonial living and cooperative breeding. Although group-defended territories are common in avian species having helpers at the nest, most of these species inhabit all-purpose territories that contain suitable sites for feeding, nesting and roosting (for reviews see refs 8, 9). Several other colonial species also breed cooperatively, but none of these defends feeding sites away from the colony¹⁰⁻¹².

We suggest that the combination of stable, defended feeding sites and shifting colony locations has contributed to the complexity of social interactions among white-fronted bee-eaters. Many authors have stressed the role of saturation of suitable territorial sites in the evolution of avian cooperative breeding^{2,8,9,13-16}. When territories are limiting, selection may favour delayed dispersal of young until suitable areas become available. Once mature young remain on the natal territory, helping might then be favoured by a variety of mechanisms¹⁷. The territory-saturation model, however, is inadequate to explain helping behaviour in colonial bird species where suitable nest sites or feeding areas are seldom, if ever, limiting.

White-fronted bee-eaters represent an intermediate case in which defensible breeding and feeding sites are spatially segregated. There is some potential for saturation of the fixed, stable feeding sites, but little for saturation of the shifting, colonial breeding sites. In contrast to most territorial cooperative species, in which ownership of a feeding territory and attainment of breeding status go hand in hand, in *M. bullockoides* the two are decoupled. Every monogamous pair in a clan is a potential breeding pair, although not all these can nest in a given season^{1,16}. A pair may occupy a high-quality feeding territory, yet show no reproductive activity one year. Alternatively, another pair might breed successfully but have a foraging

territory located sufficiently far from the active colony that the birds actually abandon their territory temporarily during the breeding season (R.E.H., unpublished data). Furthermore, a shift in feeding site need not be accompanied by any shift in breeding status or roosting site at the colony, and vice versa. Hence, there seems to be an increase in (1) the range of potential options available to each individual bee-eater, (2) the number of factors influencing the costs and benefits of each such option and, consequently, (3) an increase in the number of equivalent solutions to the problem of maximizing inclusive fitness. Given this set of circumstances, selection may favour an increase in the types and complexities of interactions among individual members of the society.

We thank the government of Kenya and the officials at Lake Nakuru National Park for permission to conduct these studies, and Irene Brown, Carolyn E. Miller and Peter H. Wrege for assistance in the field. This study was supported by the John Simon Guggenheim Foundation, the National Geographic Society, the Chapman Fund of the American Museum of Natural History and NSF grants BNS-76-81921 and BNS-79-24436.

Received 17 December 1981; accepted 14 April 1982.

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Contrast gain control in the cat visual cortex

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The eye functions effectively over an enormous range of ambient illumination, because retinal sensitivity can be adapted to prevailing light levels^{1,2}. Higher order neurones in the visual pathway are presumably more concerned with relative changes in illumination, that is, contrast, because a great deal of information concerning absolute light level is processed at the retinal level³. It would therefore be of considerable functional value if cells in the visual cortex could adapt their response levels to a steady-state ambient contrast, in a manner analogous to the sensitivity control mechanism of the retina. We have examined here the idea that adaptation of neurones in the visual cortex to ambient contrast is similar to adaptation in the retina to ambient illumination. The experiments were performed by measuring contrast response functions (response amplitude as a function of contrast) of striate neurones, while systematically adapting them to different contrast levels. Our results show that, for the majority of cortical neurones, response-contrast curves are laterally shifted along a log-contrast axis so that the effective domains of neurones are adjusted to match prevailing contrast levels. This contrast gain control mechanism, which was not observed for lateral geniculate (LGN) fibres, must be of prime importance to visual function.

Cats were prepared for single unit recording from the striate cortex by standard procedures described in detail elsewhere⁴. Anaesthesia was induced with halothane. A radial vein was

cannulated and anaesthesia was continued with sodium thiamylal while a tracheal tube and an electrode were positioned. Animals were then paralysed with gallamine triethiodide and artificially respired using 70% N₂O and 30% O₂. Sodium thiamylal was added as required to maintain anaesthesia. The electroencephalogram, electrocardiogram, body temperature and expired CO₂ were monitored. Isolated spikes from single neurones were amplified and fed into a computer for storage and analysis. Receptive fields were mapped for each unit with light bars and spots, and their ocular dominance and cell type determined using standard criteria⁵. The receptive field of the dominant eye was then aligned with the centre of a cathode ray tube screen (subtense, 30°×22°; mean luminance, 250 cd m⁻²) on which drifting sinusoidal gratings were displayed at optimal orientation, spatial frequency and drift rate.

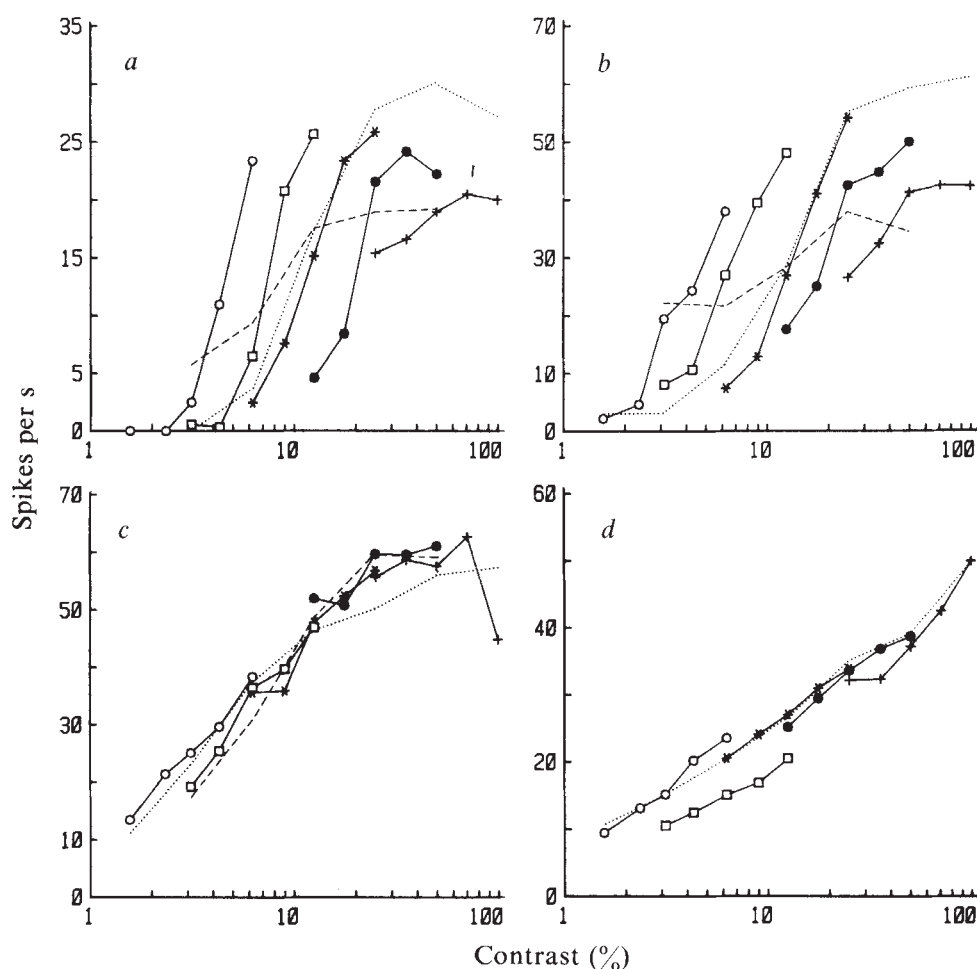
Because of the limited time available for recording from a single neurone, we used a procedure which enabled us to adapt to a given ambient contrast level while at the same time testing to obtain a contrast response function. To do this, we varied the contrast of the test grating (defined as $(I_{\max} - I_{\min}) / (I_{\max} + I_{\min})$ where $I_{\max}$ and $I_{\min}$ are, respectively, maximum and minimum intensity levels in the grating pattern) by small amounts above and below a fixed ambient level of adaptation contrast. The testing contrast levels were restricted to within ± 1 octave of the ambient contrast in order to maintain a reasonably fixed contrast level. Each contrast was presented for 4 s, a total of 10 times, and these presentations were randomly interleaved with 4 other contrasts within the small range indicated. This procedure generated a contrast response function for a particular level of contrast adaptation. The same procedure was repeated to obtain functions for other adaptation levels using different ambient contrasts. The resulting histograms were harmonically analysed to obtain d.c. and first

harmonic components as response measures. Because simple cells respond with modulated discharge to drifting gratings, the first harmonic was used for these cells, while the d.c. component was used for complex cells since they show elevated average discharge rates in response to drifting gratings^{6,7}. For LGN fibres, the d.c. component was used.

A total of 91 cells were studied in detail, of which 44 were classified as simple, 39 as complex and 8 as LGN fibres. Figure 1*a* and *b* show the results of measurements for a simple and complex cell, respectively. Curves in solid lines represent contrast response functions measured under different levels of contrast adaptation (from left to right, 3.1, 6.3, 12.5, 25, 50%). Four features of these curves are evident in both *a* and *b*. First, the curves shift along the log-contrast axis to the right as contrast increases. Second, slopes of the curves are very steep and the first four curves are approximately parallel to each other. Only at the highest contrast (>30%) is some reduction of slope found. Third, the curves span approximately the same response range (spikes per s) despite the fact that they cover considerably different input contrast domains. Fourth, even a relatively low contrast (6–10%) seems to exert a significant adapting effect, as illustrated by the first three curves from the left. These features are remarkably similar to the behaviour of retinal neurones with respect to variations of ambient light intensity. However, the operating ranges of the cortical cells are quite narrow compared with those for retinal neurones^{1,2}.

One other feature of our data should be noted. We measured the steady-state response at the ambient contrast for each contrast response function to see if the curves are centred. This is a measurement of the response level of a neurone after a sufficiently long delay following an onset of a stimulus or a step change in input levels. In other words, it is the level to which the neurone's response finally settles down when the adaptation

Fig. 1 Amplitudes of the responses of cortical neurones to drifting grating stimuli plotted as functions of contrast. Solid lines represent contrast-response functions when the neurones were adapted to different levels of contrast. The curves were measured in the order: open circle, asterisk, plus sign, filled circle, open square. The dashed curve shows the steady-state response which is derived from the last 40 s of impulse data to 80 s of continuous stimulation at fixed contrasts. We assume here that the first 40 s of data contain transient components of the response when adaptation is still in progress. The cutoff of 40 s was determined empirically and was based on our observation that most transients decayed well within this period. The dotted curve shows the contrast response when all the contrasts ranging from 1.56 to 100% were randomly interleaved. In all the following cases temporal frequency was 2 Hz. *a* Gives contrast responses (first harmonic) for a simple cell which showed substantial contrast adaptation; spatial frequency was 0.8 c deg⁻¹. *b* Gives results (d.c. component) of a similar experiment for a complex cell; spatial frequency was 0.3 c deg⁻¹. *c* Represents results from another complex cell which did not show adaptation (d.c. component); spatial frequency was 1.2 c deg⁻¹. *d* Illustrates results (d.c. component) for LGN fibre (on-centre, Y-cell) recorded in the striate cortex; spatial frequency was 0.5 c deg⁻¹.



process to a certain contrast is completed. If this steady-state response level falls in the middle of the corresponding contrast response function, one may conclude that the curve is actually centred to match the prevailing contrast level. This measurement was made before that of the corresponding contrast response function to ensure adaptation, and the results are shown as dashed lines in Fig. 1a and b. Note that these lines approximately go through the mid-points of the other contrast response functions (solid lines), establishing that the curves are, in fact, nearly centred to match the ambient contrasts.

The analogy we draw between the retinal sensitivity control mechanism for light intensity and contrast adaptation in the striate cortex is limited because all cortical cells do not show clear adapting behaviour of the type illustrated in Fig. 1a and b, while presumably, retinal neurones invariably adapt to prevailing light intensity levels. The adaptability of cortical cells to contrast varies from none to extensive. Figure 1c shows a complex cell which apparently did not adapt. All contrast response functions (solid lines) measured with different adapting contrasts and the steady-state response curve fall very close to each other, indicating fixed contrast-response properties for this unit. We found other cells, of both complex and simple types, which displayed no adaptation. One other type of unit that we recorded is of interest here. Of eight LGN afferents recorded in the striate cortex, none showed substantial adaptation. Figure 1d illustrates the results from an on-centre Y-cell from this group. As in the previous example (Fig. 1c), the contrast-response function of this LGN fibre was rigid, that is, it was not modified by adaptation. This suggests that contrast adaptation is primarily cortical in origin rather than occurring at the retinal or geniculate levels.

To facilitate comparisons, we computed an index of adaptability for each cell based on the slopes of the contrast-response functions. The index was computed by first normalizing the response and contrast ranges. By definition, the index is the mean of the slopes of the contrast response functions (solid lines in Fig. 1) minus the mean of the slopes of the curve connecting the mid-points of the functions at the adapting contrasts. For the cells we studied, the distributions of indices are unimodal and similar for simple and complex cells, with means and standard deviations of 0.52 ± 0.39 and 0.58 ± 0.41 , respectively. Although this quantification is somewhat arbitrary, it suggests that contrast adaptation is a rule rather than an exception in the visual cortex, because most cells exhibit some degree of adjustment. On the other hand, indices for LGN fibres were very low (0.17 ± 0.22), reflecting their rigid contrast-response functions.

Considered together, these results have intrinsic functional significance, but they further suggest that caution must be used in trying to specify the absolute contrast threshold of cortical neurones. Our results demonstrate that the response amplitude and contrast threshold of a striate neurone can be strongly influenced by the contrast levels the cell has experienced in the recent past, even for contrasts as low as 6–10%. Previous investigations in which absolute contrast thresholds of cortical cells have been estimated have not included controls for the effects of adaptation when measuring contrast-response functions^{6,7}. To illustrate this point, we used a procedure which was similar to that used in these other studies (all contrasts were presented in one randomized session) to generate a contrast-response function spanning the entire range. The result of this measurement is shown by dotted lines in Fig. 1a and b. Comparison of threshold estimates by extrapolation to the contrast axis shows clearly that these determinations tend to overestimate, or even possibly create, the contrast threshold, because of an overall adapting effect of the test stimuli.

We have shown that for most striate neurones, contrast adaptation behaves in a notably similar way to the retinal sensitivity control mechanism. Response-intensity curves in the retina and response-contrast curves in striate neurones shift laterally along log-intensity and log-contrast axes, respectively, so that they appropriately match prevailing input levels. We

describe this mechanism in the cortex as contrast gain control, because of the lateral displacement of the central portion of the neurone's response curve along a logarithmic axis. This is equivalent to multiplicative or divisive scaling, although we cannot specify the exact underlying mechanism. Shapley and Victor have proposed a contrast gain control mechanism for cat retinal ganglion cells⁸. The mechanism we have investigated is probably quite different because we have found that LGN fibres show little contrast adaptation. Presumably, retinal neurones would behave similarly in this respect.

One may speculate on possible functions of contrast gain control in the cortex. For example, it may provide an advantage for the maintenance of a relatively high differential contrast sensitivity. Perhaps the visual system can cope with a wide range of contrasts because of this auto-ranging capability made available by contrast gain control. Alternatively, the outputs of neurones equipped with contrast gain control might provide inputs to possible succeeding pattern processors which must be capable of extracting pattern information regardless of the contrast of images. This can be realized by matching the input domain of pattern processors with that of image contrast through contrast gain control.

This work was supported by grant EY01175 and Research Career Development Award EY00092 from the US National Eye Institute to R.D.F. G.S. was supported by training grant EY07043.

Note added in proof: None of 27 cells recorded directly in the LGN showed significant adaptation.

Received 17 February; accepted 10 March 1982.

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Hyperacuity and amblyopia

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The most frequent cause of visual loss in childhood is functional amblyopia, an abnormality of visual acuity usually associated with either anisometropia (unequal refractive errors) or strabismus (turned eye) during early development. The usual clinical investigation of the visual acuity of amblyopes involves discrimination of the high contrast letters of a Snellen chart; however, there are other aspects of acuity, for example, grating acuity (the high spatial frequency limit of vision) and Vernier acuity (the smallest perceptible misalignment). Because of the extreme precision of Vernier acuity compared with either grating or Snellen acuity, it is considered to be a form of hyperacuity which requires very precise positional information. In an effort to understand the nature of the neural abnormalities which cause the reduced acuity of amblyopes, we have measured here the Vernier acuity of amblyopic observers using an extended Vernier grating stimulus, and compared these results with their Snellen acuity and grating acuity. The results showed that different acuity losses are associated with anisometropic versus strabismic amblyopia. When scaled with respect to their grating acuity, anisometropic amblyopes, like normals, showed hyperacuity, even at high spatial frequencies, while strabismic amblyopes showed severe losses in Vernier acuity. Snellen letter acuity showed a similar deficit relative to grating acuity in strabismic but not in anisometropic amblyopes. Contrary to some previous theories which have considered that all forms of amblyopia share a common neural basis, these results strongly support the view^{1,2} that different neural losses are associated with amblyopias of different aetiologies.

The Vernier stimuli consisted of two rows of bright vertical lines on a dark background with an offset between the upper and lower rows; they are shown schematically in Fig. 1. The psychophysical paradigm was a self-paced method of constant stimuli. To obtain a criterion-free measure of the Vernier threshold a rating scale signal detection methodology was used³. Standard errors were generally 10–20% of the mean, and the per cent errors were similar in amblyopic and non-amblyopic eyes. Snellen acuity was determined using the E charts (depicted in Fig. 1) designed by Davidson and Eskridge⁴ and grating acuity was measured using the same stimuli used to measure Vernier acuity. Figure 1 shows the relationship between Snellen acuity and low spatial frequency Vernier acuity (the fundamental spatial frequency of the Vernier gratings was at least 3 octaves below the resolution limit) for non-amblyopic eyes (open circles) and amblyopic eyes (lettered circles) for each observer. The non-amblyopic eyes, and most of the amblyopic eyes, showed Vernier acuities about four to five times better than Snellen acuity. The non-amblyopic eyes and the amblyopic eyes of the anisometropic amblyopes also showed low spatial frequency Vernier acuities which were about four times better than their grating acuity (Fig. 2). In contrast, the amblyopes with strabismus showed marked departures from this linear relationship.

If the amblyopic process affected Vernier acuity and grating acuity in the same way, it might be expected that Vernier acuity would be a constant percentage of the grating resolution limit. In the anisometropic amblyopes, Vernier acuity seems to be scaled in roughly the same manner as their grating resolution. This linear scaling of Vernier and grating acuities and also Vernier and Snellen acuities in anisometropic amblyopes, is evident in Figs 1 and 2, and the statistical analysis is shown in Table 1. These findings would be predicted if the aetiology of the visual loss was due to defocused retinal imagery early in life. The results of the amblyopes with strabismus showed Vernier acuities considerably worse than would be predicted from their grating resolution. In fact, two of the strabismics

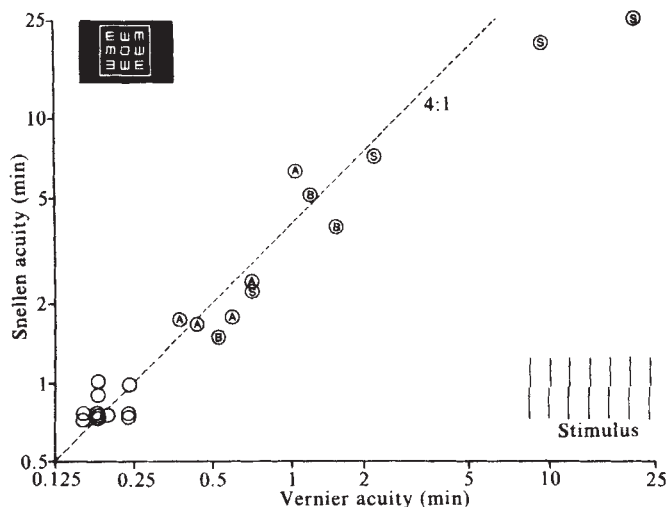


Fig. 1 Snellen acuity versus Vernier acuity (in min) for each eye of the 12 observers. The coordinates are log-log. Open circles are for non-amblyopic eyes. Circles containing A, S or B are for eyes with amblyopia due to anisometropia, strabismus and both strabismus and anisometropia, respectively. The Vernier acuity is for low spatial frequency gratings (at least 3 octaves below the resolution limit). The line represents a 4:1 relationship between Snellen acuity and Vernier acuity (that is, Snellen thresholds four times greater than Vernier thresholds). The inset on the upper left shows (in reverse contrast) the E charts used; the inset at the lower right shows the Vernier stimulus schematically. Note that the lines used were bright. Lines with random offsets were placed at the edge of the display to eliminate edge cues.

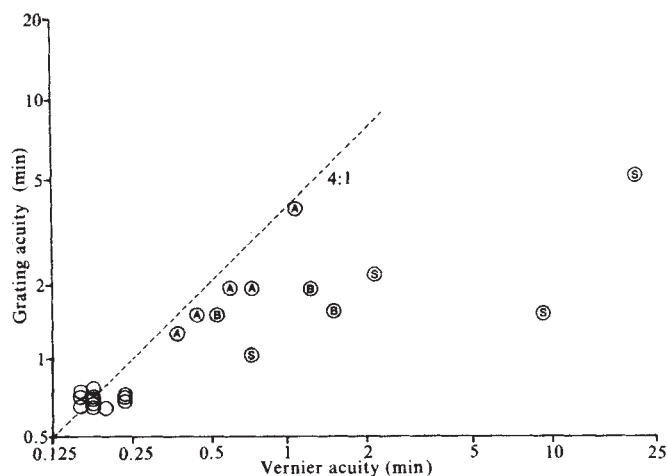


Fig. 2 Grating acuity (high spatial frequency cutoff) versus Vernier acuity (in min) for each eye of the 12 observers. Other details are as in Fig. 1. The grating acuity was determined via the method of adjustment, and the results shown are the mean of three ascending and three descending measures.

showed Vernier resolution which was two to three times poorer than their grating resolution (Fig. 2). Thus, in eyes with anisometropic amblyopia, Vernier acuity increases almost linearly with both Snellen and grating acuity (the exponents in Table 1 are close to 1.0 in anisometropic amblyopes). Snellen acuity also increases directly with grating acuity (exponent of 0.83). Strabismic amblyopes, on the other hand, show a specific loss of Vernier and Snellen acuity relative to grating resolution (the exponents relating both Vernier and grating acuity and Snellen and grating acuity are ~ 0.5). Interestingly, the relationship between the Vernier and Snellen acuities of these strabismic amblyopes more closely approximates linearity (exponent of 0.80). Thus, in strabismic amblyopia the grating acuity seems to be strongly decoupled from both Vernier and Snellen acuities. There is also evidence that cats with ablation of the striate cortex have only a mild loss of grating acuity, compared with a very dramatic loss of Vernier acuity⁵. Therefore, it may be that in strabismic amblyopia, striate cortical function (and therefore Vernier and Snellen acuity) is severely affected.

The results shown in Figs 1 and 2 were obtained at low spatial frequencies. In normal observers, Vernier thresholds are independent of spatial frequency over a wide range of spatial frequencies, increasing when the spatial frequency of the gratings is within about a factor of two of the observers' resolution (dotted line, Fig. 3). Similar results were obtained for anisometropic amblyopes (open symbols in Fig. 3); however, strabismic amblyopes (solid symbols, Fig. 3) showed a rather different result. For each of the amblyopes with strabismus, increasing the fundamental spatial frequency of the Vernier grating resulted in marked decreases in Vernier acuity from relatively high levels at spatial frequencies 4 octaves below the resolution limit, to a complete inability to perform the task for frequencies within 2 octaves of the resolution limit. In contrast, in all of the anisometropic amblyopes, Vernier thresholds remained constant up to 1 octave below the resolution limit. Thus, when scaled with respect to grating resolution, the anisometropic amblyopes are like normals in showing hyperacuity even at spatial frequencies approaching their grating resolution limit. Strabismic amblyopes, on the other hand, do not show hyperacuity at high spatial frequencies. This inability of strabismic amblyopes to maintain positional information at high spatial frequencies may be considered to be a form of 'crowding' phenomenon for Vernier discrimination⁶. Their comparable loss of acuity on the Snellen chart might be ascribed to the same deficit.

The present results cannot be explained on the basis of eccentric fixation, as the Vernier grating always included the

Table 1 Correlation coefficients and slopes of linear regression analysis of the relationships among Snellen, Vernier and grating acuity of each eye of amblyopic observers with anisometropia and strabismus

Subjects	n	Vernier versus Snellen		Vernier versus grating		Snellen versus grating	
		Correlation	Slope	Correlation	Slope	Correlation	Slope
Strabismic	14	0.98	0.80 ± 0.04	0.91	$0.42 \pm 0.05^*$	0.90	$0.54 \pm 0.07^*$
Anisometropic	10	0.94	$1.1 \pm 0.15^\dagger$	0.96	$0.94 \pm 0.09^\dagger$	0.97	0.83 ± 0.07

* Not compatible with a slope of 1 ($<10^{-10}$ chance that the data could be accounted for by a unity slope).

† Compatible with a slope of 1 (unity slope hypothesis cannot be rejected at the 5% significance level).

fovea in the stimulus field. Nor are the results readily explained on the basis of unsteady fixation, because rotating the display 90° so as to minimize the effects of horizontal fixation nystagmus, did not alter the results.

Several investigators have reported that strabismic amblyopes perceive distortions of space when viewing supra-threshold stimuli with the amblyopic eye⁷⁻⁹. If errors in spatial localization vary in time or across visual space, then tasks such as Vernier judgements at high spatial frequencies would be quite difficult. These spatial distortions seem to be primarily associated with strabismic amblyopia, perhaps resulting from abnormal binocular visual experience due to the strabismus. These results, in conjunction with the recent studies of Hess and Bradley¹, show that amblyopias resulting from different forms of visual deprivation have markedly different visual losses. While the results of Hess and Bradley suggest that anisometropic amblyopes are more handicapped in supra-threshold contrast processing, our results suggest that strabismic amblyopes are more handicapped in their ability to utilize positional information.

As Vernier acuity is such a high precision ability of the visual system, it may not be surprising that Vernier acuity should be

severely compromised by the amblyopic dysfunction. What is surprising is that Vernier thresholds at high spatial frequencies are so different in amblyopias of different aetiologies.

We thank R. Harwerth, E. Smith and H. Bedell and R. Manny for helpful discussions. This research was supported by National Eye Institute grant RO1EY01728.

Received 18 January; accepted 17 May 1982.

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Sex difference in response to alphaxalone anaesthesia may be oestrogen dependent

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The steroid anaesthetic Althesin (Glaxo), which is a mixture of two C₂₁ steroids, alphaxalone (3 α -hydroxy-5 α -pregnane-11, 20-dione—the active compound) and alphadolone acetate (21-acetoxy-3 α -hydroxy-5 α -pregnane-11, 20-dione), has been especially useful for the study of forebrain-autonomic¹ and neuroendocrine functions²⁻⁵. As determined by the loss of the righting reflex, Child *et al.*⁶ found no sex difference in the anaesthetic dose of Althesin administered intravenously (i.v.). However, in our neuroendocrine studies²⁻⁵ in which the anaesthetic was administered intraperitoneally (i.p.) and at dosage sufficient to produce surgical anaesthesia and analgesia, we observed a sex difference in the efficacy of Althesin. This may explain the difficulties that have been encountered in obtaining adequate anaesthesia (blockade of the somatomotor response to pain) with Althesin. Here we report, using cortical electroencephalography, that Althesin is a more potent anaesthetic than either sodium pentobarbitone or urethane, and that anaesthesia in the male rat requires about four times more Althesin (administered i.p.) than in the female. This sex difference is age dependent, can be abolished by administering oestrogen to the male, does not depend on sexual differentiation of the brain, and cannot be attributed to a sex difference in the metabolic clearance rate of alphaxalone. These results, taken together with those of Richards and Hesketh⁷, suggest that the effect of alphaxalone may be mediated by interactions with synaptic membranes that are more specific than simply a generalized change in membrane structure, and that these interactions are affected by sex steroids.

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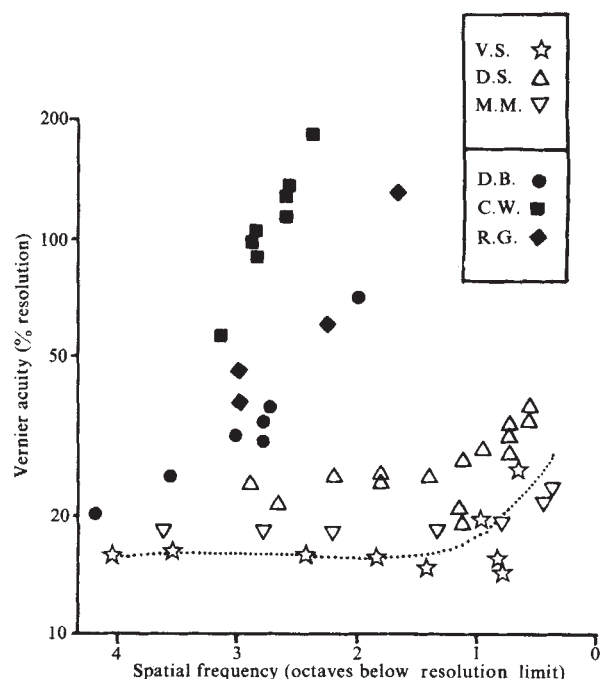


Fig. 3 Vernier acuity as a function of the fundamental spatial frequency of the grating. (The separation of the bright lines is the reciprocal of the fundamental spatial frequency of the grating.) Both the abscissa and the ordinate have been scaled to take into account each observer's grating resolution. The Vernier acuity (ordinate) is represented as a percentage of the grating resolution. The spatial frequency of the gratings (abscissa) is scaled in octaves (1 octave = 0.3 log unit) below the resolution limit. For non-amblyopic eyes, Vernier acuity is ~16% of the resolution limit for spatial frequencies an octave or more below the resolution limit (dotted line). Anisometropic amblyopes (open symbols) show similar results. The strabismic amblyopes (filled symbols) showed marked increases in Vernier acuity between 3 and 2 octaves below their resolution limit. The three strabismic and the three anisometropic amblyopes shown here have similar Snellen acuities.

All experiments were carried out on laboratory-bred Wistar rats⁴. Anaesthetics were given i.p. and the adequacy of analgesia was tested by observing the somatomotor response to painful stimuli. Duration of anaesthesia was taken as the time between the loss and the gain of the withdrawal reflex on pinching the paws or tail. This method rather than the righting reflex⁶ was used to measure potency and duration, because we wanted to determine the efficacy of Althesin as a surgical anaesthetic and analgesic. To test the potency of Althesin as an anaesthetic, female rats (200–300 g body weight) were anaesthetized with Althesin (0.5 ml per 100 g) and the changes in the cortical electroencephalogram (EEG) were recorded: 6–8 min after Althesin administration, the EEG showed periods of electrical silence with intermittent activity consisting of slow waves of high voltage, and recovery to the control pattern after 110–150 min (Fig. 1). The nature of the EEG changes was similar to that found after i.v. injection in the baboon and in man^{8–10}. The potency of Althesin was also compared with the commonly used anaesthetics by studying the effects of tail pinching and noise on the cortical EEG of female rats under Althesin (0.5 ml per 100 g), sodium pentobarbitone (4 mg per 100 g) and urethane (0.1 g per 100 g). Figure 2 shows that noise produced no significant effect on the EEG patterns for these anaesthetics. However, pinching resulted in high-frequency low-amplitude waves in the animals anaesthetized with either sodium pentobarbitone or urethane but not Althesin.

To determine whether there is a sex difference in the efficacy of Althesin as an anaesthetic, female and male rats were injected with the drug on days 17, 30, 60 and 90 after birth. The anaesthetic dose of Althesin and the duration of anaesthesia in these animals were determined. Figure 3 shows that in the female rats, there was a significant increase ($P < 0.01$) in the amount of Althesin required to induce anaesthesia at day 30 compared with that required at day 17 after birth. However,

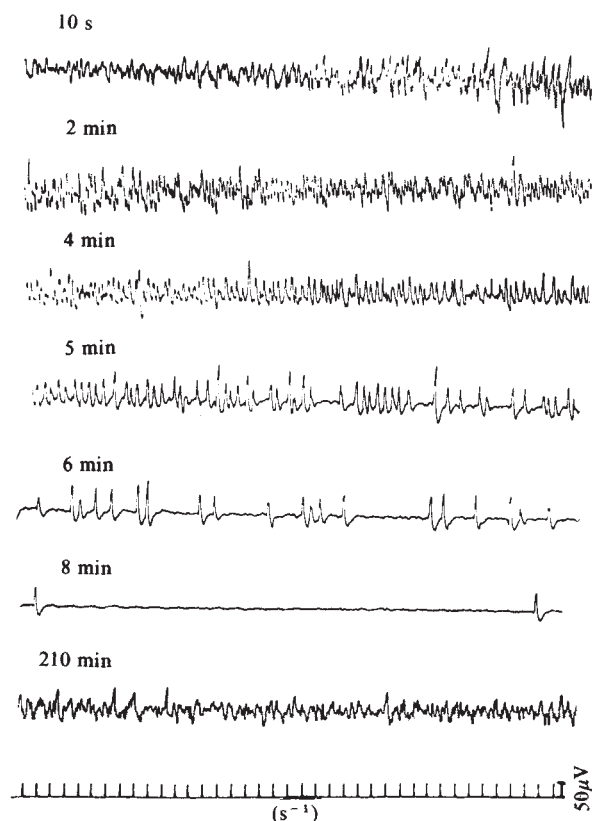


Fig. 1 A typical cortical EEG pattern observed at various times (given as shown above each recording) after Althesin anaesthesia. Bipolar stainless steel electrodes were used for recording as described by Dow *et al.*¹⁸. The EEG recording was made on a Polygraph (Grass Instrument Co.). The time and voltage calibrations are shown at the bottom.

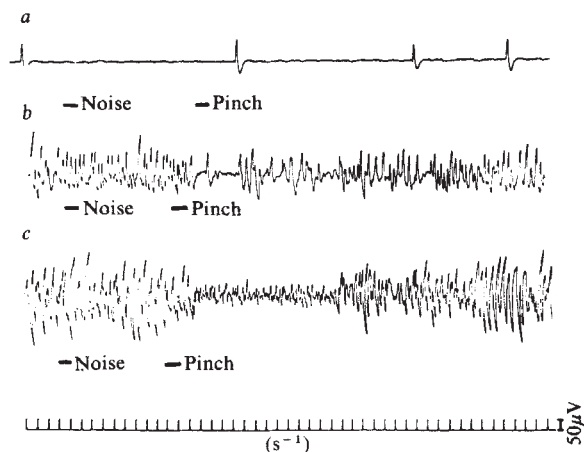


Fig. 2 Cortical EEG patterns after auditory (noise) and tactile (tail pinch) stimuli in rats anaesthetized with: a, Althesin (0.5 ml per 100 g i.p.); b, sodium pentobarbitone (4 mg per 100 g i.p.); or c, urethane (0.1 g per 100 g i.p.). The method of EEG recording was as described in Fig. 1 legend. The stimuli were given 20–30 min after the anaesthetics. The time and voltage calibrations are shown at the bottom.

after day 30, there was no significant increase in the anaesthetic dose of Althesin. The dose required in male rats was much higher than that in female rats, except on day 17 after birth. At day 90, the dose of Althesin required for anaesthesia in the male was about four times that in the female. The mean $\pm$ s.e.m. duration of anaesthesia (min) in female rats on days 17, 30, 60 and 90 was 69 ± 1 ($n = 6$), 144 ± 17 ($n = 4$), 179 ± 13 ($n = 4$) and 216 ± 18 ($n = 5$), respectively. The duration of anaesthesia in female rats was greater, but not significantly different except on day 17 ($P < 0.02$), than that in male rats of the same age [day 17, 66 ± 1 ($n = 6$); day 30, 132 ± 8 ($n = 4$); day 60, 124 ± 19 ($n = 4$); day 90, 203 ± 20 ($n = 7$)].

To determine whether this sex difference in the anaesthetic dose of Althesin is due to the changes that occur in the brain during the period of sexual differentiation, female rats were injected with testosterone propionate (TP, 1.25 mg subcutaneously (s.c.); BDH) on day 4 after birth. This procedure leads to conversion of the neuroendocrine brain from the female to the male type^{11,12}. Male rats were castrated 4 days after birth, a procedure that prevents formation of the male type of neuroendocrine brain^{11,12}. These animals and control littermates were tested for their response to Althesin on day 90. The mean $\pm$ s.e.m. anaesthetic dose of Althesin (ml per 100 g) and duration of anaesthesia (min) were 0.50 ± 0.01 and 142 ± 11 , respectively in female rats ($n = 6$); 0.59 ± 0.05 and 152 ± 23 in TP-treated females ($n = 5$); 1.99 ± 0.19 and 130 ± 15 in males ($n = 4$); and 1.87 ± 0.18 and 115 ± 3 in castrated male rats ($n = 3$).

Although alphaxalone resembles a progestogenic steroid, by using the method of VuHai and Milgrom¹³, we found that this steroid did not displace ³H-R5020 (promegestone, NEN) from cytoplasmic progesterone receptors derived from homogenates of either male or female hypothalamus. Nonetheless, it is conceivable that, like progesterone receptors in brain¹⁴, the 'receptors' for alphaxalone may be oestrogen-dependent. To test this possibility, rats were subjected to ovariectomy or castration, and some of the gonadectomized rats (2–3 weeks) were implanted (s.c.) with a Silastic capsule (Dow Corning) containing crystalline oestradiol-17 β to maintain a plasma concentration of oestradiol-17 β between 300 and 500 pg ml⁻¹ (ref. 15). Figure 4 shows that in the female rats, neither ovariectomy (2–3 weeks) alone nor ovariectomy plus exposure to oestradiol-17 β for either 3 or 10 days had a significant effect on the anaesthetic dose of Althesin. In the castrated males, however, exposure to high levels of oestradiol for 10 days reduced significantly ($P < 0.001$) the dose of anaesthetic required to induce anaesthesia (compared with that in untreated male rats) to a dose similar to that required in female rats. The duration of anaesthesia in

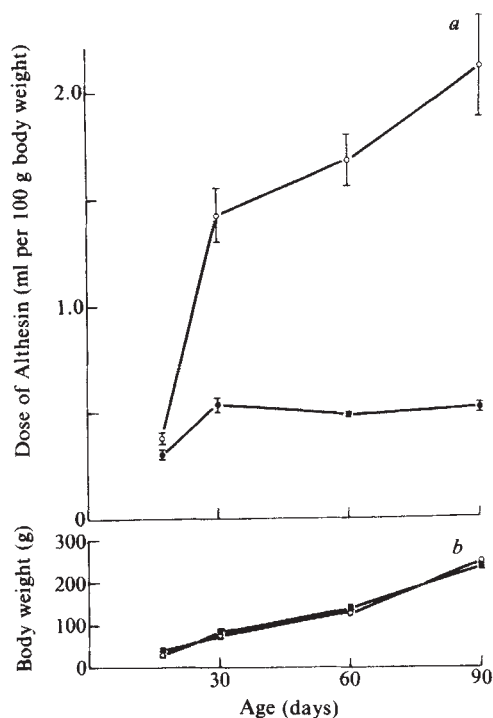


Fig. 3 Mean $\pm$ s.e.m. anaesthetic dose of Althesin (a) and body weight (b) for female (●) and male (○) rats at the various ages (shown at the bottom).

castrated male and female animals was significantly less ($P < 0.01$) than that in the intact animal.

To determine whether the sex difference in the anaesthetic dose of Althesin was due to a sex difference in the metabolic clearance rate (MCR), alphaxalone (1.2 mg per 100 g) was injected into rats (mainly 250–400 g body weight) through an indwelling intra-atrial cannula. Plasma was extracted¹⁶ from venous blood samples taken at frequent intervals after injection and assayed for alphaxalone using gas-liquid chromatography¹⁷. The MCR (litres of plasma per day, mean $\pm$ s.e.m.) was

8.0 ± 1.7 for intact males ($n = 4$); 10.2 ± 3.9 for intact females ($n = 4$); 11.2 ± 4.2 for castrated males ($n = 4$) and 10.0 ± 2.7 for castrated males treated with oestrogen ($n = 3$). Plasma alphaxalone concentrations were also measured in venous blood samples mainly taken through an indwelling intra-atrial cannula from animals injected i.p. with Althesin (0.5 ml per 100g). The concentrations rose rapidly and at 16 min after injection reached a peak ($\mu\text{g ml}^{-1}$, mean $\pm$ s.e.m.): 0.586 ± 0.037 for intact males ($n = 4$); 0.963 ± 0.189 for intact females ($n = 4$); 0.722 ± 0.140 for castrated males ($n = 4$); and 0.531 and 0.498 for castrated males treated with oestrogen. Values declined gradually after the peak but were still easily detectable at the end of the experiment, 90–120 min after injection. These data show that the significant sex difference in the effective anaesthetic dose of Althesin cannot be attributed to sex differences in the MCR of alphaxalone.

We thank Jerome E. Fink for assistance, Glaxo for providing Althesin, and the MRC and the Wellcome Trust for financial support.

Received 17 March; accepted 13 May 1982.

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Curare can open and block ionic channels associated with cholinergic receptors

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Curare has long been regarded as a typical competitive antagonist of acetylcholine (ACh) at the vertebrate neuromuscular junction. Recently, however, it has been shown that curare can also block the channels opened by ACh at the frog neuromuscular junction as well as on rat and *Aplysia* neurones^{1–4}; moreover, curare is able to depolarize rat myotubes and thus behaves as an agonist for the cholinergic receptor of this preparation⁵ (see ref. 6). Using the single channel recording technique^{7,8}, we have now found that, on rat myotubes, curare can both open and block in the same cell the channels controlled by the cholinergic receptor.

Rat myotubes were cultured at room temperature (23–26 °C) for 2–8 days after plating myoblasts taken from 18 day-old embryos. All recordings were made using the patch-clamp technique, with giga-seals formed on cell-attached patches of membrane⁸.

When the pipette contains tubocurarine (1–50 μM), single channel inward currents are observed (Fig. 1) which resemble those obtained with ACh, and which are not observed when the cells have been pretreated with α -bungarotoxin (5 $\mu\text{g ml}^{-1}$ for 10 min or more), nor when the pipette is filled only with Ringer's. A more complete analysis reveals that the curare-

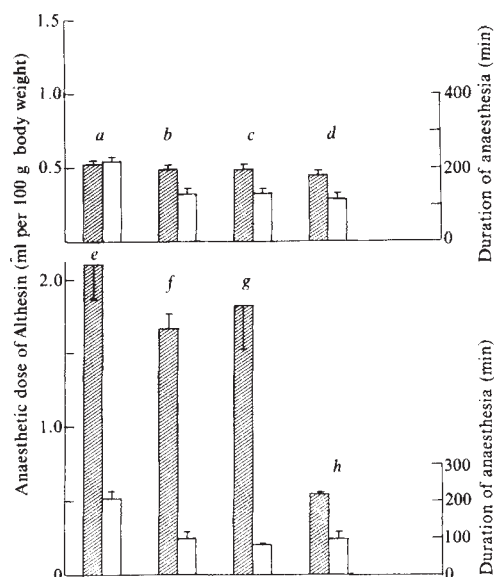


Fig. 4 Mean $\pm$ s.e.m. anaesthetic dose of Althesin (hatched bars) and duration of anaesthesia (open bars) in female (upper panel) and male (lower panel) rats with or without oestradiol-17 β treatment. Oestradiol-17 β was administered via an implanted Silastic capsule. Intact female (a); ovariectomized female (b); ovariectomized female implanted with an oestradiol-containing capsule for 3 (c) and 10 (d) days; intact male (e); castrated male (f); castrated male with an oestradiol capsule for 3 (g) and 10 (h) days.

induced channels can be separated into three populations differing by their elementary conductances. These three classes resemble the three classes of ACh-induced channels described by Hamill and Sakmann⁹ on the same preparation, and which we have also observed in a parallel study (S. Siegelbaum, A. T. and J. Koenig, in preparation).

The main population (>90%) of curare-activated channels has a conductance of ~35 pS. A second class consists of very brief channels of conductance ~50 pS (Fig. 2c), while the third group has a conductance of 10–13 pS, three times smaller than that of the modal class of channels. In the case described by Hamill and Sakmann, this sublevel of conductance was only observed at low temperature, and only after the 'full opening' of the larger channels. In the case of curare-activated channels, on the other hand, the sublevel of conductance can be observed at room temperature, and can appear separately as well as before or after a full opening (see Fig. 2).

In the patch illustrated in Fig. 2a, b, a continuous record of 184 s was examined, during which 435 events were observed, including the opening of one '50-pS channel', 373 '35-pS channels' and 43 '10-pS channels'. Moreover, in five cases a direct transition between the 10-pS and 35-pS states was seen; the inverse transition (35 pS → 10 pS) was observed in 12 cases, and in one case a double transition (35 pS → 10 pS → 35 pS). A closure of 0.5 ms between two channel openings is clearly resolved in our experimental conditions. If an apparent transition between two states of conductance resulted from mere coincidence (in time intervals <0.5 ms) in this record, the frequency of occurrence of 'simple staircases' should be 7×10^{-4} Hz, that is, two orders of magnitude less than we observed (9×10^{-2} Hz). It is thus more likely that such events reflect two states of conductance of the same channel.

The different types of channel openings observed in this cell (cell 1) and three others are summarized in Table 1. Interestingly, the frequency of occurrence of the substate increased with the curare concentration. The open time of the three classes of channel was then analysed. The rare occurrence of

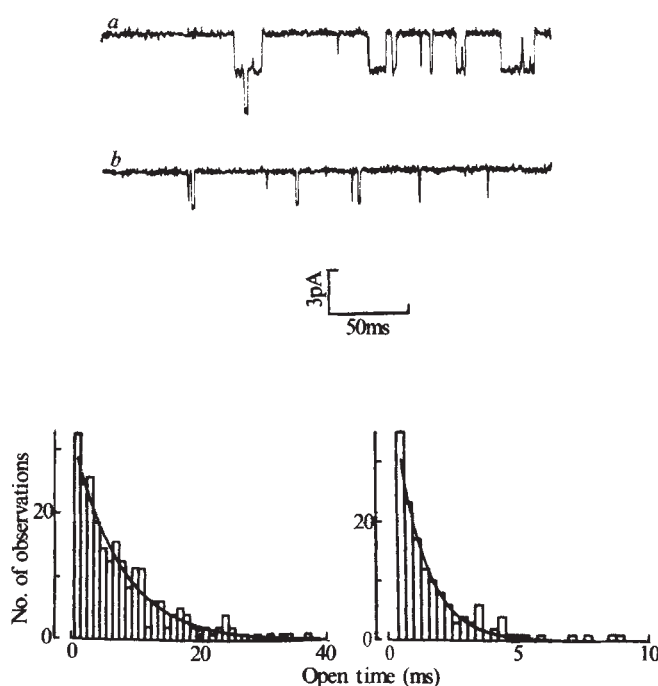


Fig. 1 Currents passing through single channels opened on rat myotubes by 300 nM ACh (a) and 20 μ M tubocurarine (b). The method of recording (cell-attached giga-seal) has been described elsewhere⁸. The bath solution contained (in mM): 115 NaCl, 2.5 KCl, 2 CaCl₂, 2 MgCl₂, 11 glucose, 5 HEPES pH 7.4. The solution in the pipette had the same composition, without glucose and plus a cholinergic ligand (ACh or curare). After these recordings had been made, the resting potentials were measured: -70 mV in cell a and -55 mV in cell b. Trace a was recorded at resting potential, and trace b with a 25-mV hyperpolarization beyond resting potential (3 kHz filtering). The opening frequency of the channels, compared with the mean frequencies obtained on other cells, indicates that both recordings were performed on hotspots (see text). The difference in the mean open time of the channels opened by ACh or tubocurarine is obvious on the records. It is further illustrated in the histograms giving the open times in the presence of ACh (left, mean = 7.3 ms) or in the presence of tubocurarine (right, mean = 1.1 ms).

Table 1 Types of current observed in four different cells in the presence of 20 μ M curare

	Cell 1	Cell 2	Cell 3	Cell 4
Length of record analysed (s)	184	120	100	12
No. of events	434	354	397	365
Type of event				
	86%	79%	88.2%	80.8%
	1.1%	1.7%	1.5%	3.6%
	2.8%	5.4%	1.5%	3.3%
	9.9%	13.3%	8.3%	10.1%
	0.2%	0.6%	0.5%	2.2%

The symbols in the left column represent the different sorts of transitions observed between the 0, 10, and 35 pS levels of conductance. The 50-pS channels were very rare (<1% of the channel openings) and are not included. For cell 4 the concentration of NaCl in the Ringer's was 30% higher than normal. In this cell, the frequency of channel openings suggests that the patch is on a hotspot.

the two 'atypical' currents prevents a systematic analysis. The data obtained indicated, however, that for these three classes, the smaller the conductance, the longer the mean open time: in two cells where there were enough of the smallest channels to obtain an estimate of their open time, in the presence of 50 μ M curare its value was two to three times greater than that of the 35-pS channels in the same cells. The mean open time of the 50-pS channels has not been estimated because these channels are both very rare and very brief (several times briefer than the 35-pS channels). The 50-pS channel illustrated in Fig. 2c is exceptionally long.

The mean open time, τ , of the main class of channels (35 pS) opened by tubocurarine was then examined in more detail. Using suberyldicholine-activated channels Colquhoun and Sakmann¹⁰ have found that histograms of both open and closed times show an excess of brief events. Similarly, we have observed that with curare-activated channels, distributions of open and closed times show several brief events exceeding that expected from a simple exponential distribution. Colquhoun and Sakmann have suggested that the very brief closed times (<1 ms) are due to transient closures rather than fast blockades of the channel. Consequently, if closures briefer than 1 ms are neglected, a τ value is obtained which is an apparent open time equivalent to that obtained using noise analysis¹⁰. Our results do not contradict the interpretation of these authors, and therefore closures briefer than 1 ms are ignored for the estimate of τ . On the other hand, according to Colquhoun and Sakmann, the excess of brief open times could be due to channels activated

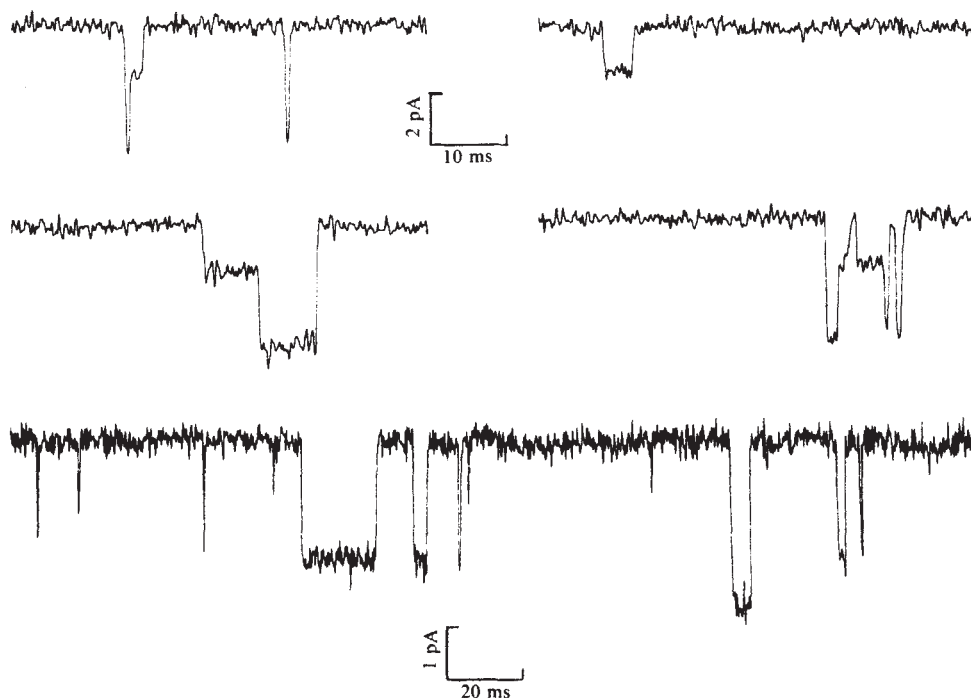


Fig. 2 Examples of the channels opened by tubocurarine. On the records obtained in a first cell (*a, b*), at a holding potential of -160 mV and in the presence of $20 \mu\text{M}$ tubocurarine, the main class of channels and the sublevel of conductance are shown. One can see that this sublevel can be observed separately as well as before and/or after a full opening. Trace *c* (another cell, -85 mV holding potential) shows the main class of channels plus one channel 1.4 times larger in amplitude. This channel has been chosen for illustrative purposes, but channels of this amplitude are usually much briefer. The holding potential is the difference between the resting potential (measured at the end of the experiment) and the potential applied to the pipette.

by a single agonist molecule. If this was the case, the excess of brief events should be more marked at low agonist concentration. In fact, with curare, the excess of brief channels is important at low concentrations of curare (for example, $1 \mu\text{M}$, not illustrated), but hardly noticeable between 10 and $50 \mu\text{M}$ (compare Figs 1 and 3). At these concentrations, the distribution of open times is compatible with the assumption that the 35 -pS channels constitute a homogeneous population. For all these reasons, supernumerary brief events were not taken into account when estimating τ .

Table 2 shows the effect of the concentration of curare on τ , measured close to the resting potential (approximately -60 mV) (S. Siegelbaum, A. T. and J. Koenig, in preparation). Increasing the concentration of curare from 10 to $50 \mu\text{M}$ results in a reduction of τ from 1.4 ms to 0.8 ms. A typical example of this effect is shown on Fig. 3. No such reduction of τ is expected or observed with a full agonist^{11,12}, suggesting that, in addition to its agonist activity, curare can act as a channel blocker, as already shown on other preparations^{1-4,13}. In a simple sequential scheme of channel block¹⁴ the extrapolation of the data obtained at 10 and $50 \mu\text{M}$ to a null concentration of curare would give a τ value of 1.7 ms.

A channel-blocking action of curare on the channel opened by curare itself becomes even more plausible when one considers the effect of voltage on τ . For a full agonist, the open time of a channel increases with hyperpolarization¹⁵, whereas a reduction of τ with hyperpolarization has been observed in the presence of various channel blockers^{16,17}. For ACh- and curare-activated channels, we measured τ at 25 mV and 75 mV beyond the resting potential and calculated the ratio of τ_{75} to τ_{25} (the values of τ for the two hyperpolarizations). For ACh-activated channels, the ratio was 1.5 (thus, as expected, an increase of τ with hyperpolarization), but, with $50 \mu\text{M}$ tubocurarine, the ratio was 0.4 – 0.8 ; thus, hyperpolarization causes a decrease of τ .

The distribution of the closed times (>1 ms) for channels activated by curare shows no marked departure from a single exponential. In other words, these channels show no bursting activity similar to that observed in the presence of a rapidly dissociating channel blocker¹⁷. If curare is a channel blocker, such a distribution is only compatible with a very slow rate of dissociation of curare from the blocked channel, as reported in other cases ($<1 \text{ s}^{-1}$, see refs 3, 13).

This conclusion is strengthened by the analysis of channels opened in the presence of ACh without or with tubocurarine.

When a high, desensitizing concentration of ACh ($10 \mu\text{M}$) is applied, silent periods (mean duration 100 – 400 ms) are observed during which all the receptors in the patch are desensitized (see also ref. 11). Between these silent periods, one channel at a time emerges from desensitization and oscillates between the

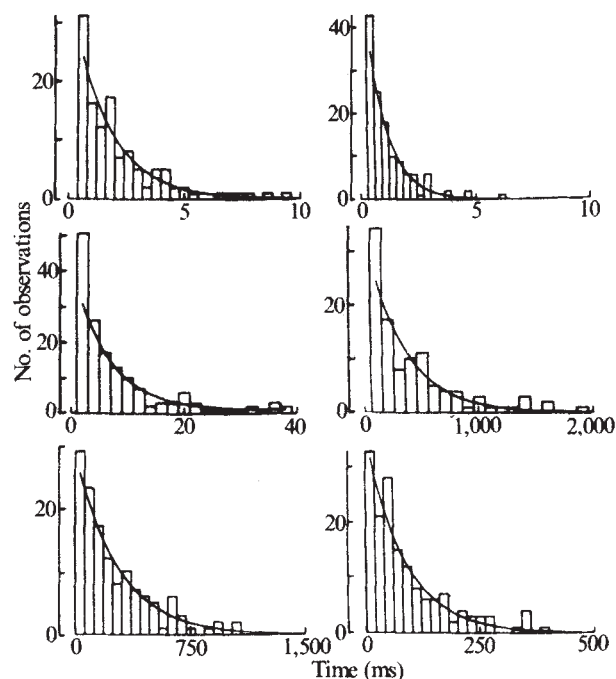


Fig. 3 Typical histograms showing open and closed time distributions, on five different cells. First row: open time distributions in the presence of $10 \mu\text{M}$ (left) and $50 \mu\text{M}$ (right) tubocurarine (two cells). The time constants are, respectively, 1.66 and 0.88 ms. Second row: closed time distribution in one cell in the presence of $10 \mu\text{M}$ ACh. Left, fast component of the distribution, shown between 1 and 40 ms. Its time constant (6.4 ms) is sufficiently different from that of the slow component (339 ms, right histogram) for subtraction of the slow component to be unnecessary in measuring the fast one. The slow component is shown for durations of 50 – $2,000$ ms. Third row: closed time distributions in the presence of $20 \mu\text{M}$ tubocurarine (left) and a mixture of $10 \mu\text{M}$ ACh and $20 \mu\text{M}$ tubocurarine (right). Both distributions (>2 ms) are reasonably fitted by one exponential. The time constants are 275 ms (left) and 83 ms (right).

Table 2 The effect of curare concentration on mean open time and opening frequency

	Curare concentration (μM)	
	10	50
τ (ms)	1.09	0.83
	1.64	0.86
	1.40	1.31
	1.66	0.46
	1	0.6
	1.29	
	1.59	
	1.38 ± 0.25	0.81 ± 0.29
Opening frequency (s^{-1})	4.4	1.7
	1.8	1
	(11.7)	0.4
	4.1	1
	5.6	(15)
	2.6	0.8
	3.7 ± 1.3	1.0 ± 0.4

Values were measured at resting potential or at a potential 25 mV more hyperpolarized. Each number corresponds to a different cell.

open and closed conformations at a high frequency (mean frequency 125–250 Hz), indicating that, at this concentration of agonist, the probability of opening is very high. When 10 μM ACh and 20 μM tubocurarine are applied together, the closed time distribution reveals only one time constant (50–100 ms, see Fig. 3), indicating that curare suppresses the bursting activity normally induced by 10 μM ACh; it also suggests that an open channel is readily blocked by curare and remains blocked for at least this duration. One can thus give an upper limit of 10 s^{-1} for the rate of dissociation of curare from the receptor.

When the frequency of opening of curare-activated channels was measured for a given concentration of curare and with similar pipettes, on different patches, two groups of values were obtained, differing by a factor of 5–20. The high frequencies, which were rare, probably correspond to receptors grouped in hot spots¹⁸. Table 2 gives the frequencies of channel opening measured in the presence of 10 and 50 μM curare. The values in parentheses, attributed to hotspots, have not been incorporated in calculating the mean. The data indicate that in the presence of 10 μM tubocurarine, the mean frequency of opening was $3.7 \pm 1.3 \text{ s}^{-1}$ ($n = 5$), but fell to $1.0 \pm 0.4 \text{ s}^{-1}$ ($n = 5$) in the presence of 50 μM tubocurarine. Thus, in this concentration range, increasing concentrations of curare paradoxically reduce the frequency of channel opening, instead of increasing it, as does ACh. This reduction is not an artefact due to the limited bandwidth of the recording system: assuming that channel openings cannot be measured when they are shorter than 300 μs , the fraction of undetected channels (in an exponential distribution) should be 20% in the presence of 10 μM tubocurarine and 30% in the presence of 50 μM tubocurarine. This difference cannot explain the observed fourfold change in the frequency of opening, but the change could result from the increase in the fraction of blocked channels. It is also possible that the fraction of activable channels could be reduced by a curare-induced desensitization. Other, more speculative explanations can be considered, in particular by taking into account the difference in the affinity of the two binding sites of the receptor for curare¹⁹.

In conclusion, for rat embryonic receptors, tubocurarine behaves like a partial agonist, with a dual (agonist and channel blocker) activity similar to the partial agonist activity of decamethonium described by Adams and Sakmann²⁰ at the frog endplate. The paradoxical action of curare on rat ganglion cells (blocking and potentiating activities¹³) might also be due to the same dual effects. It is tempting to consider that all the multiple effects of curare observed in rat myotubes, including the agonist effect, also occur on adult muscle, the only difference

being in the quantitative value of some of the rate constants. The open time of ACh-activated channels is known to be several times shorter in adult than in embryonic muscle^{21,22}, while the reduction in channel open time coincides with the disappearance of the depolarizing response to curare²³. A simultaneous shortening of the open time of ACh- and curare-activated channels could be sufficient to account for the lack of detectable current produced by curare on adult muscle. More generally, one may wonder if agonists and competitive antagonists do not constitute a continuum with only quantitative differences.

I thank J. Koenig and S. de la Porte for providing the rat myotubes, S. Siegelbaum who participated in early experiments and wrote programs for analysing the data and P. Ascher for his comments on the manuscript. This work was supported by grants from the CNRS (LA 04-295), DGRST (79-7-0785) and the Université Pierre et Marie Curie.

Received 5 March; accepted 11 May 1982.

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Mammalian neuronal actions of FMRFamide and the structurally related opioid Met-enkephalin-Arg⁶-Phe⁷

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Since the enkephalins were first isolated¹ a number of opioid peptides have been discovered, including a heptapeptide with the sequence Tyr-Gly-Gly-Phe-Met-Arg-Phe (Met-enkephalin-Arg⁶-Phe⁷)². The heptapeptide was first isolated from chromaffin granules in bovine adrenal medulla, but using immunochemical techniques it has now been identified in human, rat and bovine brains^{3,4}. The C-terminal tetrapeptide of this molecule (Phe-Met-Arg-Phe) occurs in amidated form as the molluscan peptide FMRFamide⁵. Antisera raised against FMRFamide have revealed immunoreactive material in the brains of several vertebrate species⁶, including the rat where it occurs in nerve cell bodies and terminals^{7–9}. I now report that ionophoretically applied FMRFamide has an excitatory effect on rat medullary neurones which is unaffected by the opiate antagonist naloxone. In contrast, Met-enkephalin-Arg⁶-Phe⁷ and leucine-enkephalin (Leu-enkephalin) have predominantly depressant effects, which suggests that FMRFamide acts at a separate receptor.

All the experiments were performed on male Sprague-Dawley rats (200–350 g) anaesthetized with urethane (1.2–1.8 g per kg intraperitoneally) and held in a stereotaxic frame. The cerebellum was removed by suction and micropipettes were

inserted into the medulla under visual control. Neurones were not physiologically characterized but their location and firing patterns were consistent with their being medullary reticular neurones. The brain stem was chosen for initial studies because reports from this laboratory and elsewhere have indicated appreciable levels of FMRFamide-like immunoreactivity in the area.

The results are summarized in Table 1. The predominant response to FMRFamide was excitation with rapid onset and recovery. The tetrapeptide free acid, FMRF-OH, was tested on five cells excited by the amide and had little or no effect on firing rate (Fig. 1a). The effect of Met-enkephalin-Arg⁶-Phe⁷ was variable, though mainly depressant, with a slow onset and recovery, similar to that seen with Leu-enkephalin (Fig. 1b). Naloxone antagonized the depressant action of the heptapeptide on the six cells where it was tested but had no effect on the excitatory response to FMRFamide (Fig. 1c).

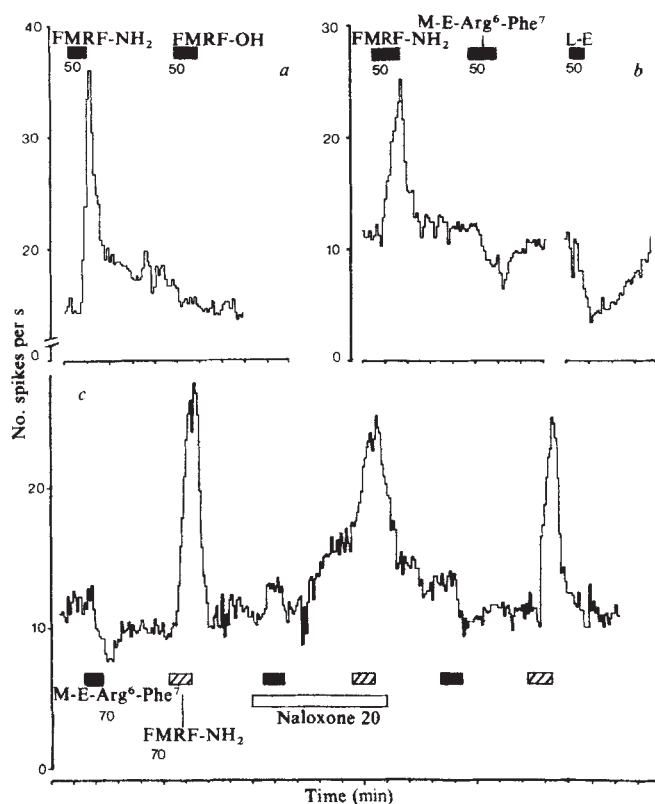


Fig. 1 Records of medullary neurone activity. Extracellular action potentials were picked up through the centre barrel of a multibarrel micropipette, amplified, fed through a window amplitude discriminator and counted over successive 5-second periods. Peptides were administered by iontophoresis as cations from the outer barrels of the pipette, using a Neurophore BH-2 iontophoresis system with automatic current balancing. Horizontal bars indicate applications; numbers under the bars are the ejecting currents in nA. Retaining currents of 5–10 nA were passed between applications. Pipettes contained combinations of the following solutions FMRFamide (2 mM, pH 5.5) Met-enkephalin-Arg⁶-Phe⁷ (0.9 mM, pH 8.5), Leu-enkephalin (10 mM, pH 5.0) (all from Peninsula); FMRF-OH (the non-amidated tetrapeptide; 1.0 mM, pH 5.5) (gift from Dr I. Galpin); naloxone-HCl (25 mM, pH 4.5) (Endo); Pontamine sky-blue (2.5% solution in sodium acetate buffer, pH 5.6) for current-balancing and marking of recording sites. *a*, Neurone excited by FMRFamide (FMRF-NH₂). The non-amidated tetra-peptide (FMRF-OH) had no effect. *b*, Different neurone to *a*, comparison of the effects of FMRFamide; Met-enkephalin-Arg⁶-Phe⁷ (M-E-Arg⁶-Phe⁷) and Leu-enkephalin (L-E). *c*, Effect of naloxone on responses to FMRFamide and Met-enkephalin-Arg⁶-Phe⁷. The depressant response to the heptapeptide was abolished while the response to FMRFamide was substantially unaltered. Naloxone itself produced some background excitation as previously observed¹².

Table 1 Summary of the neuronal responses to FMRFamide and Met-enkephalin-Arg⁶-Phe⁷

	Excited	Depressed	No effect	Biphasic
FMRFamide (39 cells)	30	1	8	0
Met-enkephalin-Arg ⁶ -Phe ⁷ (31 cells)	7	19	3	2

Nine neurones were tested with both substances and all were excited by FMRFamide and depressed by Met-enkephalin-Arg⁶-Phe⁷. The two biphasic responses seen with the heptapeptide were excitation followed by depression.

Met-enkephalin-Arg⁶-Phe⁷-like immunoreactivity has been reported in both nerve cell bodies and terminals in the rat¹⁰. It is not yet clear whether it has a neurohumoral role or is a precursor of Met-enkephalin, although its presence in terminals may be indicative of a transmitter or modulatory function. The naloxone-sensitive responses to the heptapeptide indicate that the molecule is opioid in nature, confirming previous biochemical and behavioural observations^{4,11}. However, the significant number of excitations seen means that the heptapeptide has a spectrum of actions different from that of Met- or Leu-enkephalin, which are virtually universally depressant on neurones in this area of the brain^{12–14}. In this respect, Met-enkephalin-Arg⁶-Phe⁷ is more like morphine, which also excites some brain stem neurones¹⁵.

I believe this to be the first report of FMRFamide actions on mammalian central neurones. Whether or not these actions represent a physiological function of the peptide remains to be seen. In snail neurones, FMRFamide has been observed to have both hyperpolarizing and depolarizing effects¹⁶. The C-terminal amide seems to be crucial for activity as the tetrapeptide free acid had virtually no effect; a similar finding has been reported for the octopus heart¹⁷. This inactivity also indicates that the N-terminal sequence Tyr-Gly-Gly is essential for opioid activity. There is little information available on the biosynthetic origins of the FMRFamide-like material present in rat brain. However, the fact that the amino acid sequences of FMRFamide and Met-enkephalin-Arg⁶-Phe⁷ are contained within an identified enkephalin precursor molecule^{18–20} may be significant, as it suggests that changes in post-translational processing in different cell populations could give rise to two neuropeptides with widely differing properties and hence, presumably, different functions. Regardless of the origin of the material, the results reported here, together with the immunocytochemical evidence quoted above, are compatible with a neuroregulatory role in mammals of a molecule similar to the molluscan peptide.

I thank the MRC for support and Dr G. J. Dockray for helpful discussions.

Received 8 February; accepted 11 May 1982.

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Differential expression of neurofilament triplet proteins in brain development

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Axonal transport studies and biochemical fractionation have led to the concept that the three 'triplet' proteins [approximate molecular weights 200,000 (200K), 145,000 (145K) and 68,000 (68K)] are the essential components of mammalian neurofilaments¹⁻⁵. Using a correlated biochemical and immunological approach, we have now shown that the 200K protein is under separate developmental control during rat brain differentiation and that the time of its expression differs in different regions. We were unable to detect 200K protein by immunofluorescence or in total brain filament preparations from prenatal rat brain, although the 145K and 68K proteins are both present in an apparently identical distribution. During development, progressively more 145K- and 68K-positive neurofilamentous bundles can be stained with 200K antibodies, paralleling the increasing quantities of this protein detected biochemically in brain filament preparations. We conclude that 200K protein probably has a more specialized role in neurofilament architecture and function than the other two triplet proteins.

Preparations of total intermediate filaments were obtained from rat brains of various ages by a modification of the method of Dahl *et al.*⁶ and analysed by SDS-polyacrylamide gel electrophoresis (see Fig. 1). The 200K protein was scarcely detectable on Coomassie blue-stained gels until 5 days after birth, and was still very much attenuated by day 13 after birth in relation to the quantity found in the adult. The 68K and 145K proteins were present at all stages examined. We obtained further evidence for the later appearance of significant quantities of the 200K protein using immune blots of whole brain proteins from animals of various ages (Fig. 2). A similar result can be seen in previously published gels of filament preparations from brain⁶ and optic nerve⁷ of newborn rats, though in neither case did the authors comment on it.

We were also able to document further the differential triplet protein expression during rat brain development by double-label immunofluorescence microscopy using antibodies raised in rabbit and guinea pig, each of which was specific for only one of the three triplet proteins⁸. We also used a recently described mouse monoclonal antibody specific for the 200K protein⁹. We found that this antibody performed comparably with our rabbit and guinea pig polyclonal 200K protein antisera.

Frozen sections of 5-day prenatal brain revealed numerous profiles which could be stained equivalently with 68K and 145K antibodies (Fig. 3a, b). As in adult brain¹⁰, we were unable to detect any processes that could be stained only with 145K or 68K antibodies alone. This result was observed in all specimens at all later developmental stages examined, and we conclude that the 68K and 145K proteins are invariably closely associated in each neurofilamentous bundle during the developmental sequence studied. We were unable to stain any region of the 5-day prenatal brain with any of the 200K antibodies (Fig. 3c, d). For most regions of the 1-day postnatal brain also, 200K antibody failed to stain 145K- and 68K-positive profiles, though some thicker processes in the brain stem were 200K positive (Fig. 4a, b). Neither at this stage nor later were we able to detect processes positive for 200K which were not positive, in double-label immunofluorescence, for the 68K and the 145K proteins. We examined several regions of the peripheral nervous system of 1-day postnatal animals and found that most

Fig. 1 Polyacrylamide gel electrophoresis of intermediate filament preparations from brains of variously aged animals. Lane 1, 1 day prenatal; 2, 1 day postnatal; 3, 5 days postnatal; 4, 7 days postnatal; 5, 13 days postnatal; 6, adult (about 3 months postnatal). 200, 145 and 68 indicate the position of the triplet proteins; V, the position of vimentin. The gel was loaded so that approximately equivalent quantities of 68K protein were applied. In the original gel, the 200K protein was not detectable in lanes 1 and 2, first became visible in lane 3, corresponding to 5 days postnatum. In lanes 4 and 5, slightly more 200K protein was present. Even in lane 5, however, corresponding to 13 days postnatum, the relative amount of 200K protein is much lower than that found in the adult (lane 6). Preparations were derived from the entire brain, including cerebellum and brain stem. About 1.5 g of starting material was Dounce homogenized in 20 vols of Dahl buffer⁶ (phosphate-buffered saline plus 1% Triton X-100, 0.6 M KCl, 10 mM MgCl₂, 2 mM EGTA, 1 mM EDTA, 0.5 mg ml⁻¹ tosyl-L-arginine-methyl ester-HCl, 0.15 mM phenylmethylsulphonyl fluoride, pH 7.1). After centrifugation for 10 min at 12,000 r.p.m. at 4 °C in an SS34 Sorvall rotor, the supernatant (S₁) was saved and the pellet was again homogenized with the same volume of Dahl's medium. After a further centrifugation, the supernatant (S₂) was again saved. The pellet was resuspended in 8 ml of Dahl's medium, and sonicated using a Sonifier B12 (Branson Sonic Power Company, Dahlburg, USA) at a setting of 3 W for 10 s. The material was spun in the Sorvall centrifuge as before. The supernatant was saved (S₃) and the pellet was again resuspended in 8 ml of Dahl's medium and sonicated for 10 s at 4 W. The supernatant (S₄) was collected after a further centrifugation. All supernatants were then centrifuged in the Beckman Type 40 T1 rotor at 4 °C for 30 min at 35,000 r.p.m. The pellets produced were dissolved in sample buffer and analysed on 6% analytical SDS-polyacrylamide gels. The highest yield of the neurofilament triplet proteins was found in the S₃ fractions, and these were accordingly compared. The relative quantities of the three triplet proteins detected in the other fractions of a particular preparation were similar to those found in the S₃ fraction. This gel also documents the relative decrease in vimentin expression during development. In the conditions used here, for reasons unknown, glial-fibrillary acidic protein does not co-purify with vimentin and neurofilaments, but is found predominantly in the final pellet fraction. We could, however, see a progressively relative increase in expression of this protein during development. Both findings are in agreement with previously published reports (see, for example, refs 6, 7).

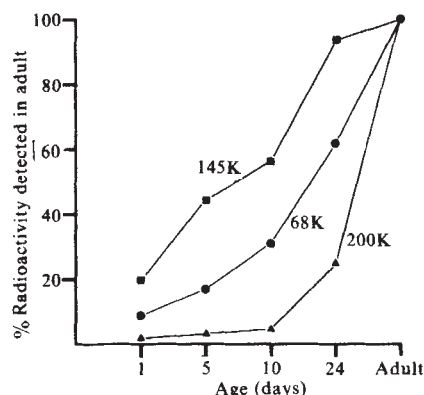
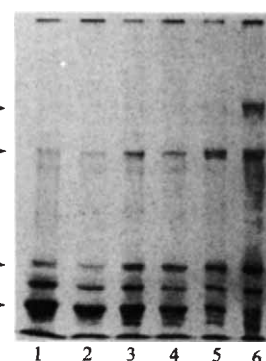


Fig. 2 Immune blotting of whole brain proteins from animals of different ages with neurofilament antibodies. Brains were homogenized in Dahl's buffer⁶ as described previously and quickly boiled in SDS sample buffer. Protein concentration was estimated using the method of Lowry *et al.*¹⁸ and equal quantities of total protein were applied to 6% SDS-polyacrylamide gels. Proteins were transferred to nitrocellulose sheets by the method of Towbin *et al.*¹⁹. The nitrocellulose sheets were stained with rabbit and guinea pig antisera strongly positive for each of the three triplet proteins⁸. Antibody staining was visualized using ¹²⁵I-labelled protein A followed by autoradiography. We quantified our results in the following manner: using heavily overexposed autoradiographs and suitable masks, we could locate the position of the radioactive protein A-antibody complex corresponding to a particular transferred triplet protein on each nitrocellulose sheet. These regions, each one corresponding to the transferred protein from a different age brain preparation, were carefully excised and the contained radioactivity estimated using a Beckman Gamma 300 counter. We used three different triplet protein antibodies from rabbit and a further three from guinea pig and obtained consistent results with different antibodies to the same triplet protein. The average results are plotted as a percentage of the radioactivity found in the adult preparations. The 200K protein clearly becomes a major component much later than the other two proteins.

nerve fibres positive for 145K and 68K protein were also positive for 200K protein (Fig. 3e, f).

Sections of most regions of the 5-day and 13-day postnatal brain showed increasing numbers of 200K-positive processes (Fig. 4c, d). However, even at these stages, few 68K- and 145K-positive fibres in the cerebral cortex were stained with 200K antibodies. We have previously reported that most, but not all, neurofilamentous bundles in the adult brain could be strongly stained with 200K protein antibodies (ref. 10 and Fig. 4e, f). However in the cerebral cortex, among other regions, some fibres positive for 68K and 145K antibodies were not strongly stained by 200K antibodies. We concluded that certain neurofilaments in the adult brain probably did not contain 200K protein, but did contain 145K and 68K protein. This conclusion is now supported by the immunological and biochemical evidence reported here for the existence of such neurofilaments in the developing brain. The combined results strongly indicate that the 200K protein is under separate developmental control from the 145K and 68K proteins, and that it appears later in

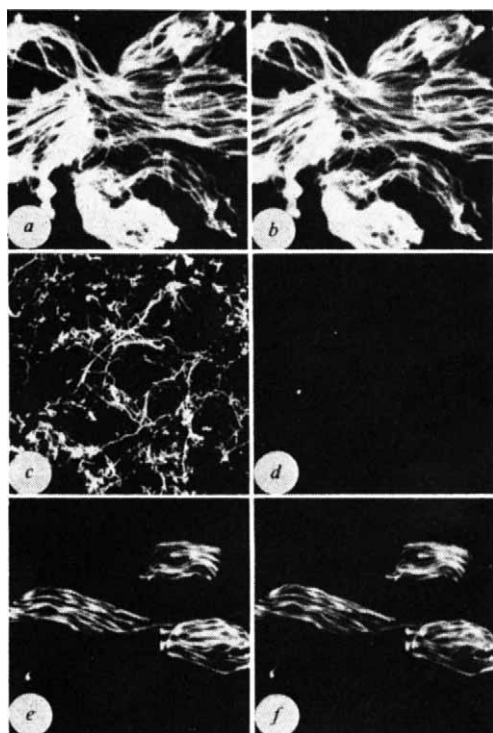


Fig. 3 Double-label immunofluorescence microscopy with individual triplet protein-specific antibodies on the developing nervous system. *a, b*, Corresponding fluorescein (*a*) and rhodamine (*b*) views of a section of a 5-day prenatal brain. *a* Was stained with rabbit 68K neurofilament antibody *b* with guinea pig 145K neurofilament antibody. The two antigens appear to have an identical distribution at this resolution. $\times 950$. *c, d* Fluorescein (*c*) and rhodamine (*d*) views of a section from a 1-day postnatal cerebral cortex. *c* Was stained with rabbit 68K antibody, *d*, with 200K monoclonal mouse antibody⁹ (compare with Fig. 4e, f). 68K antibody stains strongly, but no staining can be detected with the 200K antibody. $\times 600$. *e, f*, Corresponding fluorescein (*e*) and rhodamine (*f*) views of nerve fibre bundles in the 1-day postnatal rat tongue. $\times 380$. *e* Was stained with rabbit 200K antibody, *f*, with guinea pig 68K antibody. The distribution appears identical here, though some profiles (not shown) did not exhibit 200K staining. As with the central nervous system, all 68K-positive processes could also be stained with 145K antibodies and vice versa. All double-label immunofluorescence experiments were performed essentially as described previously¹⁰. We used the monoclonal antibody in double-label immunofluorescence using fluoresceinated or rhodaminated goat anti-mouse second antibody. Fluoresceinated goat anti-mouse was used when the monoclonal antibody was used in conjunction with a guinea pig polyclonal antibody, which was labelled with rhodaminated goat anti-guinea pig. Rhodaminated goat anti-mouse was used when the monoclonal was tested against antibodies raised in rabbit, which were labelled with fluoresceinated goat anti-rabbit antibody. Control experiments, as described previously^{8,10}, indicated no significant leakage between fluorescein and rhodamine channels in these experiments. Fluorochrome-coupled second antibodies were routinely used at dilutions of 1:20 and were purchased from Capell Laboratories with the exception of goat anti-rabbit fluorescein (Miles-Yeda).

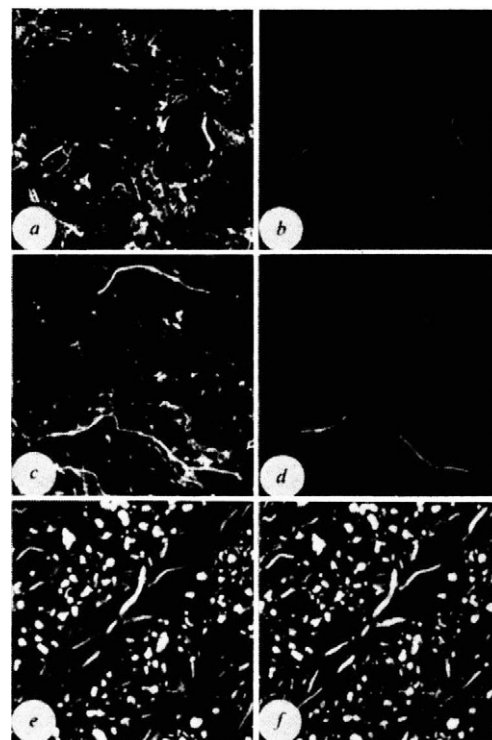


Fig. 4 Double-label immunofluorescence with triplet protein antibodies illustrating the progressive appearance of the 200K protein during development. *a, b*, Fluorescein and rhodamine views of double-labelled sections of 1-day postnatal rat brain stem. *a* Was stained with rabbit 68K protein antibody, *b*, with guinea pig 200K protein antibody. Some neurofilamentous profiles can be stained with both antibodies, but most are only stainable with 68K antibody. $\times 600$. *c, d*, Fluorescein fluorescence and rhodamine fluorescence of a brain stem region of a 5-day postnatal animal. *c* Was stained with rabbit 68K antibody, *d*, with mouse 200K monoclonal antibody. As with *a* and *b*, fibres stainable with 200K and 68K antibody are visible, though most profiles are only visible in the 68K protein channel. $\times 600$. *e, f*, Fluorescein (*e*) and rhodamine (*f*) views of a section of adult cerebellar white matter. *e* Was stained with rabbit 68K protein antibody, *f*, with mouse 200K monoclonal antibody. As reported previously, there is no detectable difference in the distribution of the triplet proteins in this region of the brain. $\times 600$.

development than the latter two neurofilament components. Furthermore, 200K is expressed in some processes before it occurs in others and ultimately becomes a component of most but probably not all neurofilamentous profiles.

In our earlier study¹⁰, we were able to identify some of the 200K-negative neurofilamentous profiles in the adult cerebral hemisphere as the dendrites of pyramidal cells. All profiles that could clearly be identified as axonal stained strongly for all three neurofilament antigens. The possibility that the 200K protein is a marker of mature axons, and may not be found in dendrites, would be consistent with the localization of 200K protein in the adult, and with the early appearance of this protein in the peripheral nervous system and brain stem. The studies of axonal transport on adult animals which originally led to the triplet hypothesis¹ and later similar studies (for example refs 2, 11) all reflect the transport of exclusively axonal neurofilaments. All published biochemical preparations of neurofilaments use adult tissue and probably select strongly for axonal neurofilaments^{3,5,12-16}. It therefore seems possible that the existence of neurofilaments composed only of the 68K and 145K proteins, perhaps dendritic in origin, has been hitherto masked by the selection for and the predominance of neurofilaments containing all three proteins. Future immunoelectron microscope studies may allow a direct evaluation of this possibility.

The results reported here raise the question of the function of 200K protein in neurofilament architecture. Our previous immunoelectron microscope study¹⁷ and that of Willard and Simon⁵ both make use of neurofilaments containing 200K protein. Both studies suggest that the 200K protein, when present,

shows a peripheral disposition and may protrude from the surface, possibly interacting with other cellular components or with neighbouring neurofilaments. The results presented here indicate that the function performed by the 200K protein is not rigidly required by every neurofilament, and is not needed at all stages of development. It should be possible to correlate the presence and absence of this protein with morphological and physiological attributes of particular neurones, and so elucidate the role of this protein in neuronal dynamics.

We thank E. Debus for providing monoclonal antibody to the 200K neurofilament protein. G. S. was supported by a postdoctoral fellowship from the Max Planck Society.

Received 8 February; accepted 18 May 1982.

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Virus persistence and recurring demyelination produced by a temperature-sensitive mutant of MHV-4

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Mouse hepatitis virus type 4 (MHV-4, the JHM strain), a positive-strand RNA virus of the coronavirus family, is well documented as an inducer of acute^{1–4} and chronic^{5,6} demyelination in mice, as well as subacute demyelination in rats^{7,8}, due to a cytolytic infection of oligodendrocytes^{3,4,7}. However, experiments to explore the role of virus and host factors in the production of chronic or recurrent demyelinating disease have been limited because MHV-4 usually produces demyelination in conditions that frequently induce a fatal necrotizing encephalomyelitis^{1–9}. To circumvent this problem, we had made and selected mutant viruses that caused both a high incidence of demyelination and a low incidence of encephalitis-induced mortality⁹. One such mutant, designated ts8, consistently caused acute demyelinating disease in over 90% of intracerebrally or intranasally (natural route of infection) inoculated, 4–5 week-old mice from several susceptible strains within 6–10 days^{9,10}. In addition, ts8 typically did not cause fatal necrotizing encephalitis, showing a low mortality (<5%)^{9,10}. This reflected a unique tropism of ts8 for oligodendrocytes, but a limited one for neuronal cells¹¹. We now report that ts8 is also useful for inducing persistent infection of the mouse central nervous system (CNS). The histopathological correlate of this infection is chronic recurrent demyelination, and virus can be demonstrated ultrastructurally in intact oligodendrocytes, in the vicinity of demyelinated areas.

BALB/c mice, 4–5 weeks old, were inoculated intracerebrally with 10^4 plaque-forming units (PFU) of ts8. Tissue homogenates from one group of mice were prepared for virus titration as 10% w/v suspensions¹². An identically treated group was killed at various time points by intracardiac perfusion with a paraformaldehyde–glutaraldehyde fixative¹¹. CNS tissue was embedded in Epon, and sections were stained with uranyl acetate and viewed with a Zeiss EM10 electron microscope¹¹. Figure 1 shows the amounts of virus found in brains of animals (five each) at 2, 4, 7, 10, 28, 60 and 365 days after inoculation. Although virus titres had declined by the seventh day after inoculation, brains from three of five mice studied at 365 days after inoculation still yielded infectious virus. Corresponding results were recorded for spinal cord tissues. All animals (25 mice) examined histopathologically 7 days after infection and later, had some degree of demyelination. Virus isolated from animals killed 1 yr after inoculation retained the temperature-sensitive growth characteristics of the initial inoculum. For example, assay of one such sample yielded 1×10^4 PFU per g brain at 34 °C, 6×10^3 PFU per g at 37 °C and <10 PFU per g brain at 39.5 °C. This sample is representative of all three isolates.

The maximal degree of acute demyelination usually corresponded directly to the amounts of infectious virus carried and

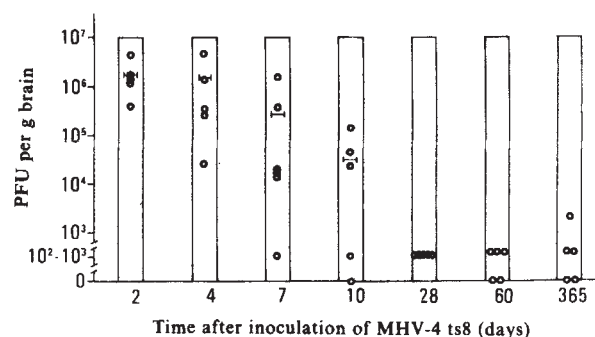


Fig. 1 Virus titres following intracerebral inoculation of 10^4 PFU MHV-4 ts8 on day 0, are expressed as PFU per g of brain tissue. Less than 10 PFU per g is indicated as zero. Brain homogenates were prepared as a 10% suspension (w/v) from each animal killed¹². Five animals were assayed at each time point at 37 °C and the bar indicates the mean virus titre. A decline in virus titre became evident 7 days after infection and reached a plateau, evident 28 days and thereafter. Virus was isolated from only three of five animals at both 60 days and 365 days after infection.

was associated with infiltrating inflammatory cells. Remyelination, characterized by thin myelin sheaths around large axonal profiles, was evident at 28 days post-infection (Fig. 2a). Thereafter, recurrent demyelination in areas that had undergone remyelination was found at 57 days (Fig. 2b) and later. The chronic demyelinating lesion was not usually associated with perivascular cellular infiltrates, although acute demyelination was associated with infiltrating inflammatory cells.

Recurrent demyelination, usually occurring in small foci, was evident at 365 days (Fig. 2c, d). Virions could be demonstrated ultrastructurally in the cytoplasm of oligodendrocytes located near sites of demyelination 1 yr after inoculation (Fig. 2e; this photomicrograph is characteristic of coronavirus virions found in the cytoplasm of several different oligodendrocytes).

Thus, this MHV-4 mutant establishes a reproducible persistent infection in mice and induces a progression from acute demyelinating disease, to a chronic recurring form. The reproducibility of this model now allows a detailed study of virus, host and genetic factors affecting virus persistence and replication in oligodendrocytes, remyelination and recurrent demyelination.

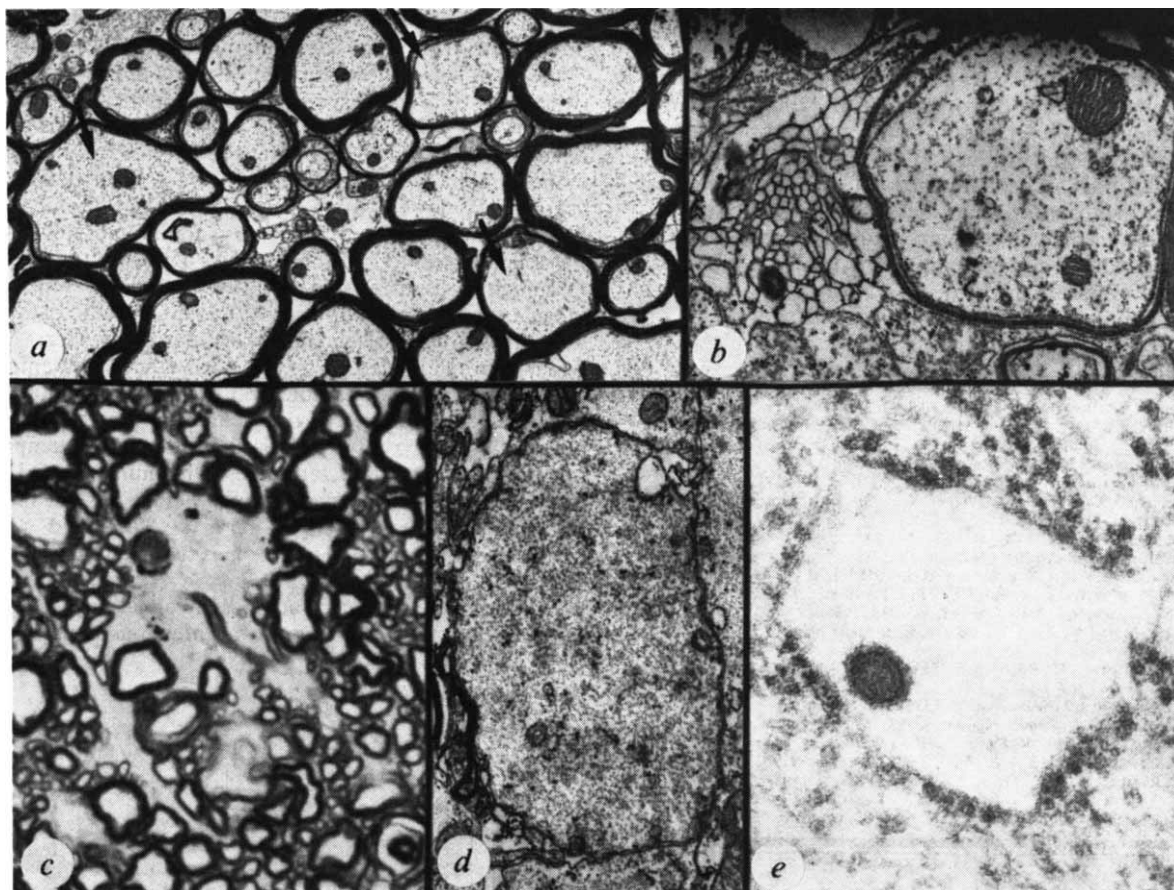


Fig. 2 *a*, Remyelinated axons were evident at 28 days after infection. They are characterized by thin myelin sheaths around large axonal profiles (see arrows). $\times 5,760$. *b*, Recurrent demyelination is evident on an axon previously remyelinated (thin myelin sheath around a large axonal profile) shown at 57 days after infection. $\times 14,400$. *c*, A small focus of demyelination surrounded by intact myelinated axons is shown in a portion of the lateral column of the spinal cord at 365 days after infection. $\times 576$. *d*, An example of a completely demyelinated axon at 365 days after infection. $\times 14,400$. *e*, An MHV virion, with its characteristic corona, is demonstrated within a cytoplasmic vacuole, in an oligodendrocyte located near demyelinated axons at 365 days after infection. $\times 108,000$.

The aetiology and pathogenic mechanism(s) of injury in multiple sclerosis, the most common demyelinating disease of man, is unknown. A large body of epidemiological and experimental evidence suggests that this disorder is due to an autoimmune and/or viral disorder¹³. The availability of the ts8 model should help to further our understanding of the pathogenic mechanism and genetic control of virus-induced acute and recurrent demyelination.

We thank Linda A. Tunison and Robert Garrett for technical assistance and Susan Edwards for manuscript preparation. This work was supported by USPHS grants NS 12428 and NS 14068. R.L.K. is a recipient of a postdoctoral fellowship from the National Multiple Sclerosis Society.

Donor origin of the *in vitro* haematopoietic microenvironment after marrow transplantation in man

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The method for long-term culture of marrow cells *in vitro* as described by Dexter¹ has recently been successfully applied to human marrow^{2,3} and is dependent on the development of an adherent stromal cell layer consisting of cells described as "endothelial-like cells, fat cells, and macrophages"⁴. The present study was designed to determine the origin and composition of the stromal cells forming the *in vitro* 'microenvironment' and maintaining haematopoiesis in long-term cultures grown from marrows of 14 patients who received marrow

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transplants from HLA identical siblings of the opposite sex. The presence of a Y chromosome was used as a marker to establish the donor or recipient origin of the cells. We found that the stromal cells became progressively donor in origin with time after transplantation and some reacted with antibody directed against factor VIII-associated antigen. In addition, donor-derived *in vitro* stromal cells synthesized both interstitial and basal lamina collagen types, indicating that the *in vitro* microenvironment is transplantable and composed in part of endothelial-like cells.

The marrow transplant procedure has been described elsewhere⁵. Clinical details of the patients are given in Table 1. Marrow aspirates were obtained between days 14 and 490 after transplantation and were used to generate long-term marrow cultures according to the method of Gartner and Kaplan² using McCoy's complete growth medium (Gibco) containing both fetal calf serum (Reheis) and horse serum (Gibco). Cultures were fed weekly with fresh medium. Although cultures of normal marrows grown in this manner can produce committed granulocytic stem cells (CFU-C) for longer than 16 weeks, the cultures were stopped at 4 weeks, a time when even suboptimal cultures have confluent stromal cell layers and produce CFU-C⁶. The supernatant cells were removed and the stromal layer was washed three times with medium, treated with 0.25% trypsin in 0.2% EDTA for 5 min, and then examined for Y-bodies⁷. In some experiments, a portion of the stromal layer was recultured, grown to confluence, and then reexamined for the frequency of Y-bodies and for the presence of factor VIII-associated antigen by fluorescence microscopy. Y-body analyses were performed on coded specimens which included normal male and female controls. The normal male stromal layers showed $66 \pm 18\%$ Y-bodies ($n = 6$) and the normal female, $1 \pm 1\%$ Y-bodies ($n = 3$). At least 200 cells were scored for each sample.

Aliquots of recultured stromal cells were tested for factor VIII-associated antigen using an affinity-purified rabbit antibody⁸. Cultured human umbilical vein endothelial cells and marrow fibroblasts were used as positive and negative controls, respectively. The stromal cells derived from transplant recipients, normal donors and normal marrow fibroblasts were radiolabelled for 24 h with L-[2,3-³H]proline and L-[³H]glycine (NEN) in the presence of β -aminopropionitrile and sodium ascorbate. Biosynthetic proteins were analysed by polyacrylamide gel electrophoresis. Collagenous proteins were identified after digestion with purified bacterial collagenase and pepsin. Electrophoresis was performed in the presence and absence of

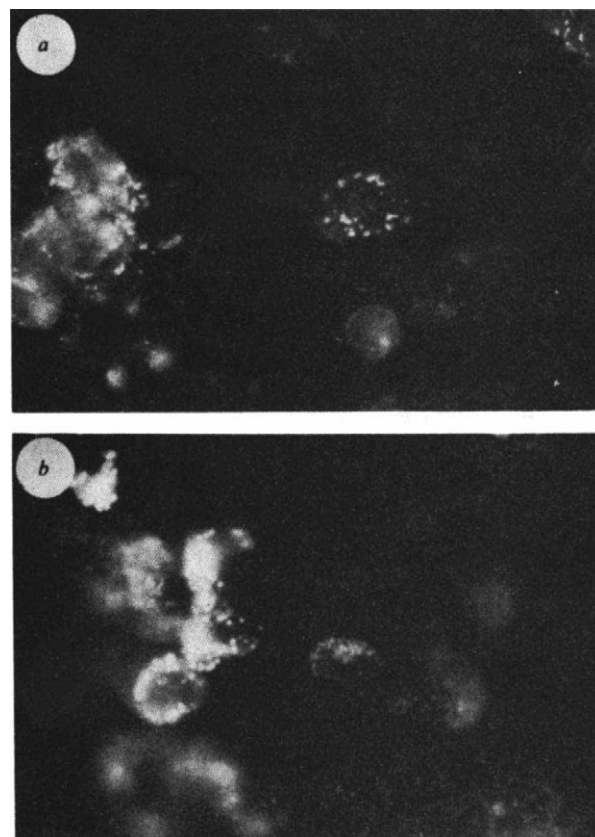


Fig. 2 Factor VIII-associated antigen. Cells were transferred onto glass cover slips. Following brief fixation in Carnoy's solution, they were incubated with affinity-purified rabbit antibody to human factor VIII antigen and then with fluoresceinated goat anti-rabbit antibody. Human umbilical vein endothelial cells (a) contain discrete cytoplasmic densities which are located in the perinuclear area. Stromal cells (b) contain patches of polygonal cells with perinuclear droplets of approximately the same size and shape as in a. Marrow fibroblasts were negative. Rabbit IgG and fluoresceinated goat anti-rabbit antibody with stromal cells produced weak nonspecific immunofluorescence. Magnification $\times 40$.

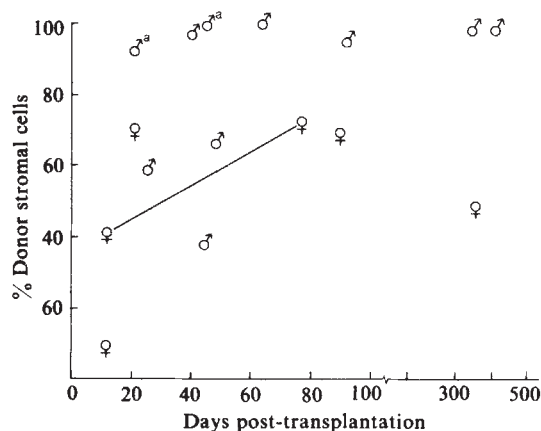


Fig. 1 Per cent of stromal cells found by Y-body assays to be of donor origin as a function of time following transplantation. The solid line connects values from sequential marrows obtained on patient 1353 on days 14 and 86 following marrow transplantation. The letter a denotes values from marrows obtained from patient 1429 on days 21 and 45 after transplantation. Female transplant recipients with 48% or greater Y-body positivity may be 100% donor-derived because normal male *in vitro* stromal cells are $66 \pm 18\%$ Y-body positive.

Table 1 Analysis of stromal cells following transplantation

Patient no.	Sex	Diagnosis	Days post-transplantation	% Stromal cells with Y-body
1353	F	CML	14	40
1443	F	ANLL	14	8
1417	F	CML	21	68
1429	M	Juvenile CML	21	8
1344	M	ANLL	25	42
1411	M	ANLL	42	2
1432	M	ANLL	43	64
1429	M	Juvenile CML	45	1
1377	M	AA	47	40
1486	M	ANLL	64	0
1340	F	ANLL	77	74
1353	F	CML	86	67
1306	M	ALL	88	5
1282	F	AA	362	48
1178	M	ALL	375	0
1108	M	ALL	490	0

The 12 patients with leukaemia were treated with cyclophosphamide before transplantation (60 mg per kg for 2 consecutive days) and total body irradiation from opposing ⁶⁰Co sources (either 200 rad daily for 6 days or 225 rad daily for 7 days). Patients 1377 and 1282 received only cyclophosphamide (50 mg per kg for 4 consecutive days). Abbreviations: CML, chronic myelogenous leukaemia; ANLL, acute nonlymphocytic leukaemia; ALL, acute lymphocytic leukaemia; AA, aplastic anaemia. One male patient was excluded from the study because of a weakly fluorescing Y-body.

dithiothreitol⁹. Transmission electron microscopy of normal 4-week old stromal cell layers was obtained with routine methods. The stromal cells were also stained for nonspecific esterase (butyrase)¹⁰.

The supernatant cells and the granulocytic colonies derived from them were shown by Y-body analysis to be totally of donor origin 25 days after transplantation. The stromal cells became progressively donor-derived after transplantation (Fig. 1). In fact, in cultures obtained from six male leukaemic patients on or after day 45 following transplantation, stromal cells from three were entirely of donor origin while the other three had 95% or more donor-derived stromal cells. The delayed disappearance of host stromal cells suggests that limited proliferative capacity may be retained following radiation. The disparity in the time required for stromal and haematopoietic cells to become entirely donor in origin is consistent with a greater radiosensitivity for the haematopoietic cells. The rate of disappearance of host stromal cells resembles that described for the loss of host alveolar macrophages following transplantation¹¹.

While the morphology of stromal cells is well described¹², their functional characteristics are unknown. Studies with mice¹³ and humans^{14,15} showed that fibroblastic cells cultured from biopsies of marrow transplant recipients were of host rather than donor origin. These studies, however, used cultures sustained with medium containing only fetal calf serum and did not allow for either the establishment of an *in vitro* microen-

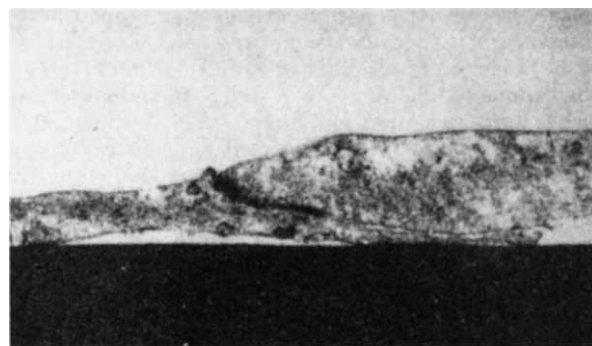


Fig. 4 Electron micrograph ($\times 10,750$) of a normal stromal cell layer showing attenuated cell processes of flattened endothelial-like cells apposed to the culture dish and joined by a prominent junctional complex. The specimen was processed using routine methods, and final sections were stained with osmium, uranium and lead salts.

vironment or the maintenance of haematopoiesis. In contrast, the stromal cells of long-term cultures obtained from three transplant recipients were entirely of donor origin. Host cell mosaicism of 2% or greater could be excluded with 95% confidence¹⁶. These observations suggest that host-derived fibroblasts of the type described^{14,15} were not present with significant frequency in our cultures. Approximately 5% of stromal cells were stained strongly positive for nonspecific esterase, suggesting that the frequency of monocytes and macrophages was also low. Three lines of evidence indicate that a component of the stromal cells has endothelial-like characteristics. (1) Donor-derived stromal cells as well as stromal cells from normal long-term cultures showed variable expression of factor VIII-associated antigen with areas of 5–25% positive cells. This was the case, for example, in stromal cells from patient 1429 at a time when 99% of the cells were donor in origin. The factor VIII-associated antigen was localized to large perinuclear granules in aggregates of flat angulated cells (Fig. 2). Marrow fibroblasts were negative. (2) Analysis of the collagenous proteins secreted by donor-derived stromal cells and normal stromal cells demonstrated the synthesis of basal lamina (type IV collagen) and interstitial collagens. This was shown in cultures from patient 1486 at a time when the stromal cells were nearly 100% donor. Marrow fibroblasts secreted only interstitial collagens in identical conditions (Fig. 3). Interstitial collagens as well as type IV collagen have been demonstrated in human marrow matrix¹⁷. Type IV collagen is known to be secreted by cultured human endothelial cells¹⁸. (3) Electron microscopy revealed the presence of junctional complexes between normal stromal cells, a feature more in keeping with endothelial cells than fibroblasts or macrophages (Fig. 4).

Thus, we conclude that the *in vitro* microenvironment in long-term cultures generated from marrow transplant recipients is donor derived and in part consists of endothelial-like cells. One of several possible interpretations of these data is that a common stem cell exists for microenvironmental and haematopoietic cells. It may be possible to test this early hypothesis^{19,20} by examining stromal layers of long-term cultures from patients with known clonal stem cell malignancies such as chronic myelogenous leukaemia to determine whether the stromal cells arise from the clonal progenitor.

A.K. is supported by a MRC of Canada Fellowship. E.D.T. is a recipient of a Research Career Award, A1 01425, from the National Institute of Allergy and Infectious Diseases. This work was supported in part by research grants CA 16448 and CA 18029 from the National Cancer Institute of the NIH and by the Veterans Administration. We thank Dr Thalia Papayanopoulou for helpful suggestions.

Received 17 August 1981; accepted 21 April 1982.

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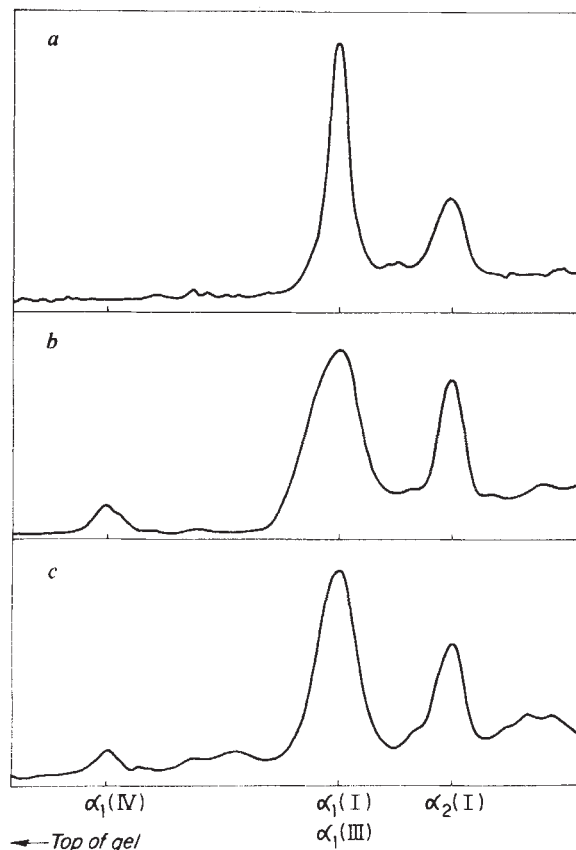


Fig. 3 Densitometric patterns of polyacrylamide gel electrophoreses from marrow fibroblasts (a), stromal cells from a normal long-term culture (b) and stromal cells from a long-term culture from patient 1486 when cells were 100% donor derived (c). Donor-derived stromal cells synthesize collagenous proteins which, following limited pepsin digestion, co-migrate with $\alpha_1(1)$ and $\alpha_2(1)$. A small amount co-migrated with $\alpha_1(1)$ and retained interchain disulphide bonds, a characteristic of type III collagen. Another collagenous protein retained interchain disulphide cross-links following pepsin digestion. This protein migrated with an apparent molecular weight of 170,000. These features suggest that these chains are derived from type IV collagen. The ratio of $\alpha_1(IV)$ to $\alpha_2(1)$ chains was similar in normal and donor derived cultures. Marrow fibroblasts failed to secrete type IV collagen *in vitro*.

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I region-restricted antigen presentation by B cell-B lymphoma hybridomas

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The activation of T lymphocytes for many responses^{1–4} requires interaction with specialized antigen presenting cells (APC) which express I region associated (Ia) antigens. Cells of the monocyte-macrophage lineage⁵ and the recently described dendritic cell⁶ have been shown to be effective APC. Antigen-specific T cells appear to recognize a complex of antigen and Ia molecules on the surface of the APC and such joint recognition is the basis of the restriction of T-cell activation due to the major histocompatibility complex⁷. The Ia molecules appear to be *Ir* gene products^{8–10}. However the biochemical nature of antigen presentation has been difficult to approach largely because of problems in obtaining sufficient numbers of highly purified APC. Although several Ia antigen-positive B lymphoma cell lines, which have proved very effective APC, have been recently described^{11,12}, they have all been of the same *H-2^d* haplotype. We report here on hybridomas produced by fusion of normal C57BL/6 (B6) B cells (*H-2^b*) to a hypoxanthine guanosine phosphoribosyl transferase (HGPRT) deficient variant of one of these lymphoma cell lines. The hybridomas appear to express functional Ia molecules of both parental cell haplotypes. Such cloned somatic cell hybrids should prove useful in studying the properties of *Ir* genes and Ia molecules in antigen presentation.

Hamano *et al.*¹³ have recently prepared an HGPRT deficient variant from one of the stimulatory BALB/c lymphoma lines, M12.4, by selection in the presence of 6-thioguanine after mutagenesis with ethyl methanesulphonate. This HGPRT-

Table 1 Phenotype of B-cell lymphoma lines and B cell-B lymphoma hybridomas

Designation*	Cell-surface markers						
	IgM	I-A ^d	I-E/C ^d	H-2K ^d ,D ^d	I-A ^d	$\beta_{AE\alpha E}^d$	H-2K ^b ,D ^b
M12.4	—	+	+	+	—	NT	NT
M12.4.1	—	+	+	+	—	—	—
TH 2.2	+	+	+	+	+	+	+
TH 2.5	+	+	+	+	+	+	+
TH 4.3	+	+	+	+	+	+	+

Nt, not tested, but anticipated, on genetic basis to be negative.

*M12.4 is a BALB/c B lymphoma cell line; M12.4.1 is a cloned HGPRT-deficient variant derived from M12.4; TH 2.2, TH 2.5 and TH 4.3 are cloned lines of independent somatic cell hybrids produced by fusing LPS-induced blasts of B6 B cells with M12.4.1.

deficient subline (M12.4.1) has been cloned and somatic cell hybrids between it and lipopolysaccharide (LPS)-stimulated normal B cells from B6 (*H-2^b*) mice have been produced. The resulting hybridomas exhibit typical surface markers of B6 origin including surface IgM, I-A^b antigens and H-2K^b, D^b antigens as well as the surface markers Ia^d, I-E/C^d and H-2K^d, D^d of the BALB/c parental line. The phenotype of three of these hybrids (TH 2.2, TH 2.5, TH 4.3) and the parental lines is shown in Table 1.

To establish that the ability of these hybridomas to stimulate mixed lymphocyte response (MLR) and to present antigen derives from products of genes encoded in their I^b as well as their I^d region, three approaches were taken. First, the ability of one of these hybridomas, TH 2.5, to stimulate both BALB/c (*H-2^d*) and B6(*H-2^d*) responder T cells in allogeneic MLR was assessed. Table 2 shows that as few as 10³ TH 2.5 cells, which are of the *H-2^{b,d}* haplotype, stimulated the proliferation of both BALB/c and B6 responder T cells, but not of (B6 × BALB/c)F₁ T cells (BCF₁ T cells). The BCF₁ T cells could, however, be stimulated by allogeneic B10.A spleen cells. M12.4, the parental BALB/c B lymphoma line, stimulated proliferation by B6 but not BALB/c T cells. This suggests that the TH 2.5 cell line expresses Ia antigens encoded in both the I^b and I^d subregions.

To establish that such hybridomas used gene products of the I-A^b subregion as restriction elements, we tested the ability of cells of the TH 2.2 line to present the IgG2a hybridoma protein,

Table 2 TH 2.5 stimulates an allogeneic MLR by BALB/c and B6 T cells

Stimulators	Type	Cell no.	³ H-thymidine uptake (c.p.m.)					
			Responder cell population					
			Expt 1		Expt 2			
None			BALB/c	BCF ₁	B6	BALB/c	BCF ₁	
			392	347	285	292	204	
BCF ₁ spleen		3 × 10 ⁵	29,685	753	38,288	10,334	452	
BALB/c spleen		3 × 10 ⁵	752	1,048	47,861	822	757	
TH 2.5		3 × 10 ⁴	82,984	2,527	15,004	95,736	2,267	
		10 ⁴	60,456	0	15,651	50,743	1,254	
		10 ³	17,513	83	1,251	—	—	
M12.4		10 ⁵	3,209	NT	11,765	—	—	
B10.A		3 × 10 ⁵	NT	NT	NT	14,934	13,164	

3 × 10⁵ nylon wool column-passed spleen cells from BALB/c (*H-2^d*), BCF₁ (*H-2^{b,d}*) or C57BL/6 (*H-2^b*) donors were co-cultured with the designated stimulator cells at 37°C in 5% CO₂ for 5 days in RPMI 1640 containing 2.5% human serum, L-glutamine, penicillin-streptomycin, 2-mercaptoethanol and HEPES buffer. 16 h before collection, 1 μCi of ³H-thymidine (NEN) was added to each well. TH 2.5 and M12.4 cells received 10,000 R and spleen stimulator cells 2,000R from a caesium source before addition to culture. ³H-thymidine uptake by irradiated tumour or spleen cells, cultured alone, has been subtracted from the c.p.m. shown. Backgrounds of tumour cells cultured alone were less than 5,000 c.p.m.

15–5–5 (ref. 14), to T cells of a long term B10.A(5R) line (ref. 18) specific for this antigen. This tests the expression of *Ir* gene function by TH 2.2 cells because the response to this IgG2a protein is controlled by an *Ir* gene which maps to the I-A subregion (S. H. & R. H. S., manuscript in preparation). Table 3 demonstrates that as few as 10³ TH 2.2 hybridoma cells elicited substantial proliferation from the IgG2a-responsive T-cell line while the drug-marked parent line, M12.4.1 and control BALB/c spleen cells were unable to do so. TH 2.2 cells were far more potent in eliciting T-cell proliferation to IgG2a than conventional splenic APC, as 10³ TH 2.2 cells yielded a greater response than 5 × 10⁵ irradiated spleen cells.

The hybridoma cell lines also expressed functions of the hybrid Ia molecule, $\beta_{AE\alpha E}^d$, generated by the pairing of the AE_β chain of *b* haplotype and the E_α chain of *d* haplotype¹⁵. This was demonstrated by showing that these hybridomas could present a cytochrome *c* peptide to a T-cell hybridoma specific for that peptide in conjunction with the hybrid Ia molecule as a restriction element. A synthetic cytochrome *c* peptide consisting of amino acids 88–104 found in tobacco horn worm moth (moth) cytochrome *c* was used. The antigen-specific T cell hybridoma was prepared by fusing T cells from moth cytochrome *c*-primed (B10 × B10.A)F₁ donors with the AKR

Table 3 TH 2.2 presents the IgG2a hybridoma protein to a long-term T-cell line

Antigen-presenting cells		Response to 15-5-5 (³ H-thymidine uptake; c.p.m.)		
Type	Cell no.	No antigen	15-5-5 (50 µg ml ⁻¹)	Δ
None	—	166	229	63
BCF ₁ spleen	5 × 10 ⁵	2,215	13,395	11,180
C57BL/10 spleen	5 × 10 ⁵	130	8,241	8,111
BALB/c spleen	5 × 10 ⁵	380	588	208
M12.4.1	10 ⁴	2,947	3,984	1,057
	10 ³	1,692	1,158	—
TH 2.2	10 ⁴	1,635	56,832	55,197
	10 ³	289	45,636	45,347

A long-term B10.A(5R) T-cell line was prepared by the method of Kimoto *et al.*¹⁸. It was specific for the IgG2a hybridoma protein 15-5-5 in association with I-A^b subregion gene products. 15-5-5 is of the *Igh-Id* allotype and possesses κ light chains¹⁴ (gift of Dr David Sachs). The T-cell line was rested for 2 weeks without antigen. These cells (10⁴) were then co-cultured with the designated APC for 4 days in a medium consisting of a 1:1 mix of RPMI 1640 and (Eagle's Hank's amino acids) containing 10% fetal calf serum plus the supplements listed in Table 2. Δ Refers to the difference in c.p.m. obtained in the presence and absence of 15-5-5.

thymoma BW5147 (refs 16, 17). The response of B10.A(5R) cells to moth cytochrome *c* is controlled by complementary *Ir* genes mapping to I-A^b and I-E/C^d (ref. 17). All three hybridomas (TH 2.2, TH 2.5 and ThH 4.3) presented moth cytochrome *c* better than BCF₁ spleen cells (Table 4). This presentation was antigen and Ia specific, as TH 2.5 did not present antigen to a B10.A hen egg lysozyme specific T-cell hybridoma whose restricting element, I-A^k, it does not possess. M12.4.1 cells, B6 spleen cells and BALB/c spleen cells all failed to present this antigen. Cytochrome *c* presentation by the B-cell hybridomas could be blocked by the addition to the culture of a monoclonal antibody (clone 17.3.3, a gift of Dr David Sachs) directed against the hybrid Ia molecule, thus confirming its role in antigen presentation (data not shown).

It is therefore clear that hybridomas produced from the fusion of normal B6 LPS-activated spleen cells with a BALB/c B lymphoma line have gained the functional properties of B6 Ia

Table 4 B cell-B lymphoma hybridomas present a cytochrome *c* peptide to a T-cell hybridoma

Antigen-presenting cells		³ H-thymidine uptake (Δ c.p.m.)		
Type	Cell no.	Expt 1 Cytochrome <i>c</i> hybridoma	Expt 2 Hen egg lysozyme hybridoma	Cytochrome <i>c</i> hybridoma
None	—	374	0	0
BCF ₁ spleen	5 × 10 ⁵	10,033	0	8,428
B6 spleen	5 × 10 ⁵	—	0	961
BALB/c spleen	5 × 10 ⁵	—	166	0
M12.4.1	10 ⁵	0	—	0
	10 ⁴	0	—	0
	10 ³	0	—	—
TH 2.2	5 × 10 ³	20,003	—	35,061
	10 ³	10,479	—	—
TH 2.5	5 × 10 ³	21,657	99	36,310
	10 ³	12,168	0	—
Th 4.3	10 ⁴	30,797	—	14,252
	10 ³	11,902	—	—
B10.A spleen	5 × 10 ⁵	—	14,127	—

A tobacco horn worm moth (moth) cytochrome *c* specific T-cell hybridoma was prepared by fusing secondary *in vitro* stimulated lymph node T cells from a cytochrome *c*-primed (B10×B10.A)F₁ donor with the T-cell lymphoma BW5147. A cloned subline of one such somatic cell hybrid which secreted interleukin-2 when co-cultured with antigen in the presence of antigen-presenting cells which express the β₂μA^d hybrid Ia molecule was selected for these experiments. The hen egg lysozyme (HEL) hybridoma was prepared similarly by fusing HEL primed lymph node T cells from a B10.A animal with the BW5147 thymoma. 1 × 10⁵ T hybridoma cells were co-cultured with the designated APC in the presence or absence of the antigen, a synthetic moth cytochrome *c* peptide composed of amino acids 88-104 (gift of Drs D. Hansburg, T. Fairwell and E. Appella, NIH) or hen egg lysozyme (gift of Dr Eli Sercarz). After 2 days of culture, supernatants were collected and assayed for interleukin-2 activity by ³H-thymidine uptake on HT-2 cells, an interleukin-2 dependent T-cell line developed by Dr James Watson and provided by Dr Phillipa Marrack. Results are expressed as c.p.m. obtained in the presence of antigen minus c.p.m. obtained without antigen (Δ c.p.m.).

molecules and *Ir* gene products because (1) they stimulate BALB/c responder T cells in an MLR, (2) they present IgG2a, the response to which is controlled by an *Ir* gene in the I-A subregion, to a long-term antigen-specific I-A^b restricted T-cell line, and (3) they present a cytochrome *c* peptide, the response to which is determined by the combinatorial product of genes mapping to the I-A^b and I-E^d subregions, to an antigen-specific T-cell hybridoma. Most recently, successful somatic cell hybridization between the M12.4.1 and normal B cells from (BALB/c×A/J)F₁ mice has been performed. These hybrids both express H-2^a determined *Ir* genes and the I-A^k antigens donated by the A/J parental cell.

Cloned B lymphocyte somatic cell hybrids, when used in concert with long-term continuous T-cell lines or cloned antigen-specific T-cell hybridomas, provide an elegant model system for studying the genetic and structural properties of *Ir* genes and Ia molecules in antigen presentation. In particular, by use of specific Ia mutants, B-cell hybridomas should provide tools to confirm the identity of *Ir* gene products with Ia antigens and to examine the structural basis of specificity in antigen presentation.

E.H.-K. is supported by a fellowship from the CRI.

Received 26 January; accepted 18 May 1982.

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Redistribution of intermediate filaments during capping of lymphocyte surface molecules

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Intermediate filaments (IF) constitute a major cytoplasmic filamentous network of higher eukaryotic cells that is distinct from actin and myosin microfilaments or microtubules. Although structurally similar, these filaments are formed by chemically and antigenically different proteins. Vimentin is the major IF polypeptide of mesenchymal cells and cultured non-mesenchymal cell lines¹. Recently, we have characterized a monoclonal IgM antibody from a patient with Waldenström's macroglobulinaemia which is directed against vimentin². Using this monoclonal antibody, we have shown by direct immunofluorescence that intermediate filaments of human B and T lymphocytes consist of vimentin. In cells exposed to colcemid, the intermediate filaments retracted into a juxta-nuclear aggregate ('coil') characteristic of vimentin filaments. As most components of the cytoskeleton, especially actin³ and myosin⁴, have been implicated in the capping phenomenon, we investigated the effect of capping of either β₂-microglobulin or

membrane immunoglobulins on the organization of the intermediate filament network. We report that capping of these surface molecules induced the redistribution of vimentin just beneath the cap. When colcemid-treated cells were allowed to cap, the location of the cap always coincided with the coil, suggesting that the anchorage point of intermediate filaments is situated within the uropod.

Intermediate filaments of human lymphoid cells were stained by direct immunofluorescence using a human monoclonal IgM (IgM_{DUV}) antibody specific for vimentin polypeptides. We have shown previously that IgM_{DUV} stains only cells and tissues expressing vimentin IF and reacts with a single protein of molecular weight (M_r) 57,000 (57K) from cell extracts and triton-insoluble cytoskeletal material in immunoblotting experiments². The activity of IgM_{DUV} is removed by purified vimentin. IgM_{DUV} did not react with actin, tubulin or the other four classes of IF polypeptides, that is, keratins, desmin, the neurofilament triplet and glial fibrillary acidic protein.

IgM_{DUV} stained blood lymphocytes with a perinuclear rim which was revealed to be a filamentous network encompassing the nucleus (Fig. 1a); this network was more conspicuous on lectin-stimulated lymphoid cells. All blood B (surface immunoglobulin (sIg)-positive cells) and T lymphocytes were positive. Several human lymphoblastoid cell lines belonging to the T (3 cases) or B (5 cases) cell lineage were also positive, with a well-developed filamentous reseau (Fig. 2a). As expected for vimentin IF, colcemid-treated cells showed a characteristic coil which appeared as tiny filamentous aggregates located at one pole of the cell in blood lymphocytes (Fig. 1b) and as a single distinct ring in blast cells from the KE₃₇ T-cell line (Fig. 2b).

Capping of blood lymphocyte surface immunoglobulin with rhodamine-conjugated anti-Ig antibodies induced a reorganization of vimentin intermediate filaments—they assembled just underneath the cap into a diffuse aggregate (often devoid of detectable filamentous structure) as revealed by the subsequent staining of cells with fluoresceinated IgM_{DUV} (Fig. 3). B cells which did not cap retained their filamentous network of vimentin filaments, as did T cells. Similarly, capping of β_2 -microglobulin molecules at the surface of either blood lymphocytes or leukaemic cells from the T-cell line KE₃₇ led to a redistribution of vimentin intermediate filaments.

This phenomenon was examined further on splenic mouse B lymphocytes (IgM_{DUV} activity is not species-specific). When cells were allowed to cap for 2, 5, 10 and 30 min, the redistribution of vimentin IF was clearly observed at each stage of the capping, that is, from the clustering of surface immunoglobulin on one half of the cell surface to the uropod state. Within 5

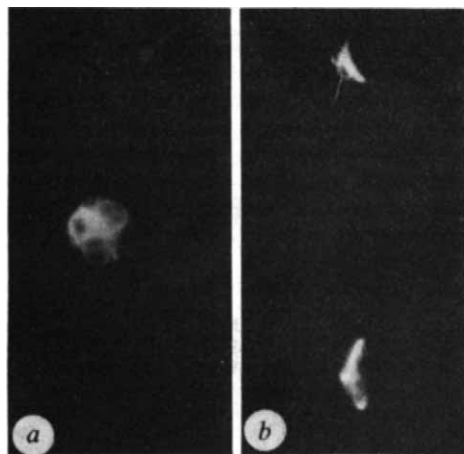


Fig. 1 Vimentin network indicated by fluoresceinated conjugated IgM_{DUV} at a concentration of $80 \mu\text{g ml}^{-1}$. Cells were fixed with 2% formaldehyde at 37°C for 10 min and permeabilized in 1% Triton X-100 for 1 min. *a*, Peripheral blood lymphocytes; *b*, colcemid-treated blood lymphocytes (cells were cultured in 10% fetal calf serum, RPMI medium with $20 \mu\text{g ml}^{-1}$ colcemid for 24 h and then processed as described above).

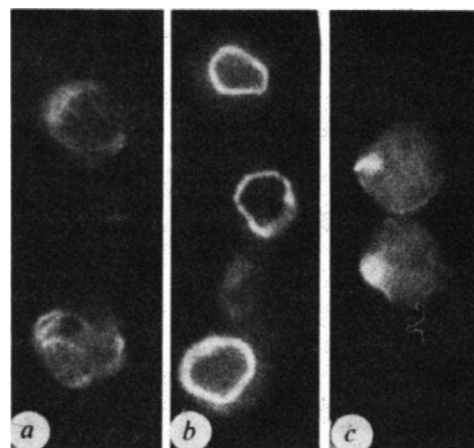


Fig. 2 Organization of vimentin intermediate filaments in KE₃₇ cells, revealed by fluoresceinated IgM_{DUV} as described in Fig. 1 legend. *a*, Filamentous network of untreated cells; *b*, ring-like coil of colcemid-treated cells; *c*, vimentin patch of cells after capping of surface β_2 -microglobulin (cells were incubated with a mouse monoclonal antibody to human β_2 -microglobulin, provided by Dr S. F. Schlossman, washed, further stained by a goat anti-mouse IgG serum and allowed to cap at 37°C ; the reaction was terminated by addition of 2% paraformaldehyde at various times).

min, >80% of B lymphocytes had capped and exhibited redistributed intermediate filaments. The filaments accumulated in the periphery of the cytoplasm of the small round lymphocyte, always in close contact with surface immunoglobulin. Essentially identical results were observed for the capping of β_2 -microglobulin on KE₃₇ T cells examined at various capping times. Note that the reorganization of intermediate filaments after capping seemed to differ from that after colcemid treatment; this was most striking in KE₃₇ cells which had a ring-like coil after colcemid treatment whereas a small patch of vimentin was observed after capping of β_2 -microglobulin (Fig. 2b, c respectively).

As colcemid does not inhibit the capping phenomenon of lymphoid cells⁵, we investigated the spatial relationship between the location of blood lymphocyte sIg or β_2 -microglobulin caps and that of vimentin IF coils and found that the caps were always located precisely above the coil. These results demonstrated that the anchorage point of the coil is not random within the cell, as the location of the cap at the cell surface corresponds to the uropod, a highly specialized part of the cell⁶.

Thus, it can be concluded that intermediate filaments participate in the capping phenomenon as do other cytoskeletal components such as actin, myosin and tubulin. That actin has a major role in the capping, probably by generating contractile motile forces, is shown by the inhibition of capping by cytochalasin B, which disrupts the physical arrangement of microfilaments⁵. The role of microtubules is less obvious as depolymerization of tubulin by colcemid has no effect on the rate and extent of capping⁵. This drug may actually enhance ligand-induced capping^{6,7} and lead to a ligand-independent capping of some T-lymphoma surface antigens⁸. The mechanism of these latter phenomena and the possible role of intermediate filaments have to be re-evaluated in the light of the present findings.

Finally, the recently demonstrated physical or functional connections between the three main cytoskeletal networks^{9,10} may provide a clue as to the co-capping of actin, myosin, tubulin and intermediate filaments with surface proteins. One method of investigating the role of intermediate filaments in capping may be to elucidate the nature of the interaction between anti-immunoglobulin cross-linked sIg and triton-insoluble cell material as demonstrated recently by Unanue¹¹. Our preliminary data show that this material contains both actin and vimentin.

The role of intermediate filaments remains largely unknown, although it has been suggested that they play a part in the

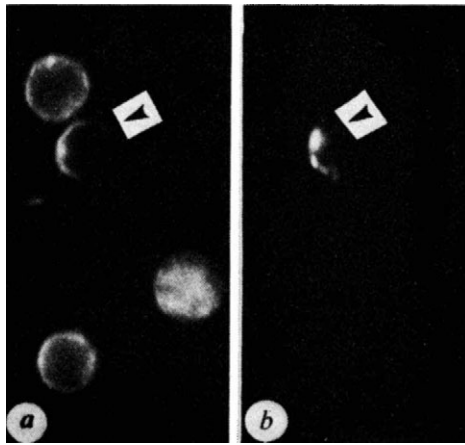


Fig. 3 Double labelling of normal blood B lymphocytes for surface immunoglobulin and vimentin filaments. Cells were first stained with rhodamine-conjugated F(ab')₂ fragments of rabbit IgG to μ chain, washed and allowed to cap. The cells were then fixed and stained for vimentin as described in Fig. 1 legend. Controls with fluoresceinated IgM having no anti-IF reactivity were negative. *a*, Staining for vimentin; the arrow indicates polar redistribution of vimentin in a B cell with redistributed surface immunoglobulin; *b*, same field of view showing surface staining with rhodamine-conjugated anti- μ antibodies.

mechanical integration of various cytoplasmic organelles¹. The very close relationship between the redistribution of surface molecules and that of lymphocyte vimentin suggests a novel function for intermediate filaments.

This work was supported by grant 81.1029 from INSERM.

Received 22 February; accepted 13 May 1982.

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Expression of mouse immunoglobulin genes in monkey cells

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The generation of functional immunoglobulin light-chain genes from variable (V), joining (J), and constant (C) gene segments has been elucidated recently^{1,2}. The structural features which regulate the expression of immunoglobulin genes, however, have not been well defined. To further our understanding of immunoglobulin gene expression, African green monkey cells (CV1), HeLa cells, and mouse L cells were transfected with κ -chain gene-containing plasmids. We report here that efficient transient expression occurred in the CV1 and HeLa systems when the gene was placed, in a recombinant plasmid, under simian virus 40 (SV40) promoter control. This is the first report of immunoglobulin gene expression in a eukaryotic gene transfer system. No significant expression of the κ -chain genes under the control of their own promoters was detected, however, in systems in which, for example, the β -globin gene is expressed from its own promoter^{3–5}. Possible reasons for this difference are discussed.

Recombinant plasmids were constructed which contained, in different positions, the functional κ -chain gene of myeloma T, called T1 (refs 6, 7), or the aberrantly joined, but expressed, MPC 11 Lf gene^{8–10} (see Fig. 1). Four of the plasmids had the κ -chain genes inserted in the direction of late, two in the direction of early SV40 transcription. The coding region of the T1 gene is located close [31 base pairs (bp)] to the SV40 sequences with their promoters in pTKp6 alone; in the other plasmids there are 0.8 and 3.0 kilobases (kb) of mouse DNA (with the putative promoters) upstream of the T1 and the MPC-11 Lf gene, respectively.

In pTKp6-transfected CV1 cells (transfection was done as described in ref. 11), a distinct transcript was detected having the size of the light chain mRNA from myeloma T, indicating correct splicing of the primary transcript (Fig. 2). Standardized blot hybridization experiments (not shown) indicated that the content of specific κ -chain mRNA in total cellular RNA of pTKp6-transfected CV1 cells was 30–40% that of myeloma T cells. Transfection of CV1 cells with the other plasmids led to patterns lacking the distinct transcript band (see Fig. 2; similar patterns were obtained with pTKp1, the MPC 11 plasmids, and with pBR322). From DNase and RNase digestions, the hybridizing material consists mainly of partially degraded plasmid DNA, but it is possible that it also contains

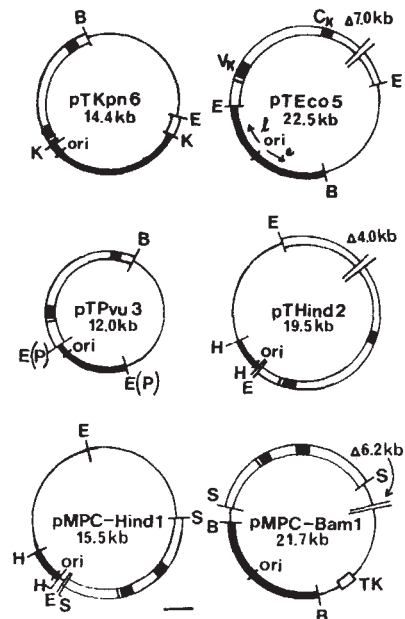
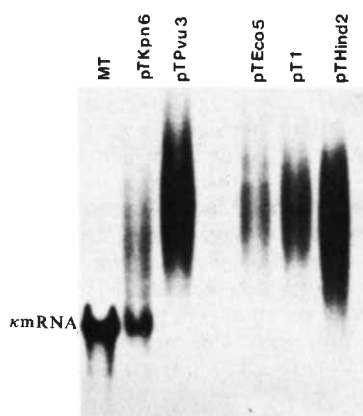


Fig. 1 Construction of recombinant plasmids. pTKp6, the 5' *EcoRI*/*BamHI* fragment (5.5 kb) of fragment T1 (ref. 6) was ligated to the large *EcoRI*/*BamHI* fragment of pBR322; the resulting plasmid pT1 was cleaved with *KpnI*, treated with alkaline phosphatase, and ligated with *KpnI*-cleaved SV40 DNA; the clone having the opposite SV40 orientation is called pTKp1. pTEco5, a plasmid (donated by U. Hänggi) consisting of the large *EcoRI*/*BamHI* fragments of pBR322 and SV40 DNA cleaved with *EcoRI*, treated with phosphatase, and ligated to the 14-kb *EcoRI* fragment T1. pTPvu3, *EcoRI* linkers (Collaborative Research) were added to *PvuII*-cleaved SV40 DNA; the origin-containing fragment (isolated by electrophoresis on a low-melting point agarose gel) was cloned into the *EcoRI* site of pT1. pTHind2, the *HindIII* origin fragment of SV40 was ligated with *HindIII*-cleaved pBR322 DNA and the clone having the *EcoRI* site close to the origin was chosen (pHind ori); the *EcoRI* fragment T1 was introduced into this site. pMPC-Hind1, the MPC-11 Lf gene^{8,9}, cloned as a 6.5-kb *SstI* fragment¹⁰ in λ gtWES (donated by H. Schnell), was isolated as a 10-kb *EcoRI* fragment and ligated with *EcoRI*-cleaved, phosphatase-treated pHind ori DNA. pMPC-Bam1, the TK gene plasmid²⁶ (donated by C. Weissmann) was partially cleaved with *BamHI*; full-length linear molecules were isolated, phosphatase-treated, and ligated with *BamHI*-cleaved SV40 DNA. One of the orientational isomers was chosen and cleaved at the indicated *SstI* site into which the 6.5-kb MPC 11 Lf fragment was ligated (other orientational isomer pMPC-Bam20). The structure of all recombinants was verified by restriction analysis. Enzymes were prepared in our laboratory (*EcoRI*, ligase, *BamHI*, *HindIII*) or obtained from Boehringer-Mannheim (phosphatase) and BRL (*KpnI*, *PvuII*, *SstI*). ■□, Immunoglobulin gene fragments, coding and non-coding regions, respectively; —, SV40; —□—, TK plasmid²⁶, only in pMPC-Sst1; —, pBR322 and, in pMPC-Hind1, also λ phage. B, *BamHI*; E, *EcoRI*; E(P), *PvuII* site converted into an *EcoRI* site; H, *HindIII*; K, *KpnI*; S, *SstI*.

Fig. 2 Analysis of RNA from transfected CV1 cells and myeloma T. Total cellular RNA was isolated as described elsewhere²⁷; and samples (10 µg) were glyoxylated²⁸, electrophoresed on 1.5% agarose gels and blotted on to nitrocellulose filters²⁸, which were hybridized with nick-translated pT1 DNA. Autoradiographs were exposed for a day with an intensifying screen:

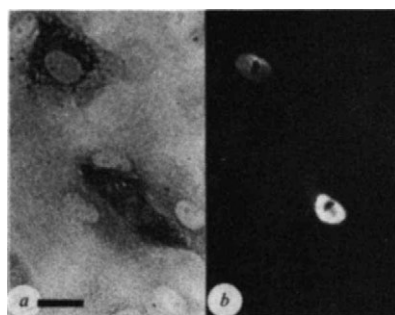


some unprocessed or partially processed transcripts. Of the amount of κ mRNA produced in the pTKp6-transfected cells, 0.5% would have been detected in the cells transfected with the other plasmids.

Screening for translation products in pTKp6-transfected CV1 cells using anti- κ chain and anti-T-antigen antibodies gave positive results with 8–10% of the cells (Fig. 3); >90% of the T-antigen-positive cells were κ chain-positive. In addition, pTKp6-transfected HeLa cells were positive for both antigens. In cells transfected with pTKp1, pTEco5, pMPC-Bam1, and pMPC-Bam20, only T-antigen expression was detected, while the cells transfected with the other plasmids were negative for either antigen.

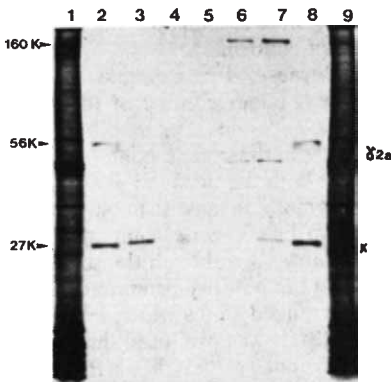
The translation product of the pTKp6-transfected cells that was precipitable by anti- κ antibodies was identified electrophoretically; cells transfected with three other plasmids were negative (Fig. 4). The product in the pTKp6-transfected cells is probably the mature κ chain; the fact that it migrated just above the reference κ chain (Fig. 4) may be due to gel loading effects. We cannot exclude incomplete processing as a reason for this slower migration but this is unlikely because other translation products are efficiently processed in CV1 cells^{12,13}.

Fig. 3 Detection of κ light-chain cross-reacting material in transfected CV1 cells by use of enzyme linked immunoadsorbent assay (ELISA) (a) and by indirect immunofluorescence (b).



For ELISA staining¹⁷, CV1 cells grown on coverslips were fixed for 2 s in 0.9% NaCl, 10 mM phosphate, pH 7.4 (PBS) containing 3.5% formaldehyde and 0.2% Nonidet P-40 (NP40), then for 10 min in PBS/formaldehyde. After washing twice for 5 min in PBS, rabbit-anti-mouse κ -chain antiserum (Miles) in a 1:20 dilution was layered over the cells. Incubation in a humid chamber for 30 min was followed by two washes with PBS. Peroxidase-coupled pig anti-rabbit immunoglobulin antibodies (Boehringer) were added in a 1:80 dilution. Incubation and washing were as before. The cells were incubated for 10 min in the ELISA substrate mix¹⁷ for development of the red colour. The reaction was stopped by PBS washing. For T-antigen staining, the ELISA-stained cells were immersed for 2 min in PBS/1% NP40 followed by two PBS washes. Anti-T-antiserum (given by W. Deppert) was preadsorbed on methanol-fixed CV1 cells and a 1:20 dilution was added to the cells. Incubation and washing were as before. Fluorescein isothiocyanate-coupled rabbit anti-mouse immunoglobulin was used as second antibody. After PBS washing, the cells were observed under a fluorescence microscope (Leitz). In a, CV1 cells transfected with plasmid pTKp6 were ELISA-stained 30 h after transfection. The dark cytoplasmic staining (red) indicates κ -chain determinants which are absent from neighbouring cells. In b, the same cells are photographed under UV illumination, green fluorescing nuclei indicating the presence of SV40 T-antigen. Scale bar, 25 µm.

Fig. 4 Identification of ^3H -leucine-labelled κ light chains in transfected CV1 cells; 30 h after transfection, 5×10^6 cells were incubated with $50 \mu\text{Ci ml}^{-1}$ ^3H -Leu in Leu-free medium (Gibco) for 3 h. Cells were collected and lysed in 250 µl PBS, 0.1% NP40, 1 mM phenylmethylsulphonyl fluoride by four freeze-thaw cycles. The 10,000g supernatant (30 min, 4 °C) was incubated



with 20 µl of goat anti-mouse κ -chain antibodies (Byk) for 2 h at 4 °C. This material was adsorbed on to 125 µl of a 10% protein A-Sepharose (Pharmacia) suspension in PBS for 2 h at 25 °C. The protein A beads were then washed three times (10 min each time) with either PBS, 0.1% NP40 or PBS without detergent. Note that only the detergent-washed beads show non-specifically bound proteins (upper two bands in lanes 6 and 7). The beads were boiled in SDS sample buffer²⁹ and subjected to polyacrylamide gel electrophoresis on a 10–16% gradient gel²⁹. Fluorography was done using ^3H -Enhance (NEN) according to the supplier. Lanes 1, 9, ^3H -Leu-labelled nuclear proteins from CV1 cells (K, molecular weight in thousands, determined from stained gels); 2, 8, secreted immunoglobulins of myeloma T, κ and $\gamma 2a$ chains; 3, 7, proteins from 1×10^6 cells transfected with pTKp6, washed without and with NP40, respectively (no radioactive κ -chain band was seen when normal goat serum was used instead of goat anti- κ antiserum or when mouse κ chains were added for competition); 4–6, proteins of 1×10^6 cells transfected with pT1 (4), pTEco5 (5) and pTPvu3 (6, NP40-washed).

To examine for expression of the cloned mouse genes in a mouse cell system, L cells (thymidine-kinase defective, TK⁻) were transformed¹⁴ with the plasmids of Fig. 1 and the herpes TK gene^{15,16}; (the TK gene was not added to pMPC-Bam1, pMPC-Bam20 or to pMPC-Bam1 analogues lacking the SV40 moiety). Screening of permanent TK⁺ clones for κ -chain and T-antigen determinants was negative throughout. Several clones were also assayed for κ -chain mRNA, with negative results. Transformation with large amounts of pTKp6 and pTPvu3 in the absence of carrier DNA yielded only a few TK⁺ L-cell clones which were, however, κ chain-negative. The clones which had been obtained by transformation with recombinant λ phages containing the T1 gene¹⁸ were negative in the assays of Figs 3 and 4. In summary, no κ -chain expression was detected in the L-cell system. Also, no transient expression was observed in pTKp6-transfected L cells, probably due to problems associated with the ELISA staining of these cells.

The efficient transient expression of a κ -chain gene under SV40 promoter control, as described here, should be a useful tool in further experiments on immunoglobulin gene expression, for example in the study of transcript processing and translation. As for the question of immunoglobulin gene regulation in immunocompetent cells, it is more interesting that no κ -gene expression under the control of its own promoters was found in several recombinant plasmids.

Somatic cell genetics suggests that immunoglobulin gene expression is *cis*-controlled¹⁹. The putative promoters (or activators) and transcription initiation sites of the two κ genes studied here are as yet unknown, but they may be located close to the start codon. In some κ -chain mRNAs, the 5' untranslated regions were found to be very short^{20–22}. For the T1 and MPC-11 Lf genes^{7,9} this would mean that the TATA and CCAAT sequences located within 110 bp upstream of the ATG codon contribute to the promoter function. In pTKp6 these sequences are replaced by SV40 sequences whereas in the other plasmids of Fig. 1, they are present together with other potential promoter sequences. Such sequences were found further upstream in several V genes (including T1; see ref. 7) and also in the unrearranged but transcribed C gene²³. Below we suggest

three possible reasons for the lack of expression under 'own' promoter control.

(1) Because, for example, the rabbit β -globin gene is expressed, under control of its own promoter, transiently in HeLa cells^{3,4} and also in permanent L-cell clones⁵, certain sequences in the κ gene-containing fragments may in some way interfere with the production of stable transcripts. (2) Alternatively, something may be missing from our systems. It is known that germ-line V-gene segments are not transcribed (not even in myelomas²⁴), although the sequences upstream of the genes including the putative promoters are the same as in the respective rearranged genes which are transcribed (for example, see refs 7, 25). It was proposed that both V and C segments have to be present to form a functional chromatin domain²⁴. We

may extend this by saying that for the κ genes studied here, the formation of such a domain and, with that, expression, requires DNA sequences further upstream and/or downstream than those present in our plasmids. Such sequences may mediate a certain nucleosome arrangement or other features of the chromatin structure which facilitate transcription. (3) Another possibility is that in immunoglobulin gene expression, some as yet undefined factors provided in tissue-specific differentiation events may have a role. The best way to distinguish between the three possibilities and to achieve expression under own promoter control may be to establish a gene transfer system with immunocompetent cells.

This work was supported by Bundesministerium für Forschung und Technologie and Fonds der Chemischen Industrie.

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A cloned cell with NK function resembles basophils by ultrastructure and expresses IgE receptors

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Natural killer (NK) cells are defined by their ability to lyse certain tumour cells *in vitro* without previous exposure to them^{1–5}, and have been postulated as effectors of immune surveillance against spontaneous neoplasms⁶. Because they kill some non-neoplastic lymphoid cells, they may also have a role in immunoregulation^{7,8}. NK cell activity resides in a small proportion of normal mouse spleen cells (<5%) that have been difficult to characterize completely. They may represent a heterogeneous group of effector cells whose precise relationship to other myelopoietic or immunological cells has remained obscure^{9–12}. We have previously described a cloned mouse cell line (Cl. Ly 1² NK-1⁺/11) with the functional characteristics of natural killer cells activated by interferon or other factors⁸. We now find that this cloned line, like basophils^{13,14} and mast cells¹⁵, expresses high-affinity plasma membrane receptors (Fc_εR) specific for IgE antibody. In addition, the clone contains cytoplasmic granules similar by ultrastructure to those of basophils of the mouse and other species. Our findings indicate that cells sharing morphological and biochemical features of basophilic granulocytes can mediate NK lysis.

Cl. Ly 1² NK-1⁺/11 lyses the YAC-1 lymphoma 100 times more efficiently than unfractionated spleen cells, but does not lyse the NK cell-resistant RL-12 lymphoma⁸. Like NK cells activated by interferon or other factors, it lyses the EL-4 lymphoma, the P815 mastocytoma, and lipopolysaccharide-activated B lymphocytes⁸. This clone expresses the Thy 1 and Ly 5 glycoproteins characteristic of T lymphocytes and other

haematopoietic cells, as well as the Qat 4, Qat 5 and NK-1 surface antigens characteristic of certain NK cells⁸. Although similar to some T lymphocyte clones which may mediate NK lysis⁸, Cl. Ly 1² NK-1⁺/11 expresses no Lyt 1 or Lyt 2 glycoproteins by immunofluorescence. It differs from mature macrophages because it adheres poorly to plastic and does not phagocytose antibody-coated erythrocytes. Cl. Ly 1² NK-1⁺/11 and at least 10 other clones with the same pattern of NK lytic activity are identical by light microscopy⁸. All these clones contain prominent cytoplasmic granules similar to those of certain uncloned rat¹⁶ and human¹⁷ NK cells.

By transmission electron microscopy¹⁸ the cytoplasmic granules of Cl. Ly 1² NK-1⁺/11 cells (Fig. 1a) were identical to those of mouse basophils (Fig. 1b). Large round electron-dense cytoplasmic granules such as these have previously been considered a unique characteristic of basophils: they have been identified in the basophils of several mammalian species including man and murine rodents but not in other granulocytes, lymphocytes, macrophages, promonocytes or mast cells¹⁹.

Because of its morphology, we evaluated whether Cl. Ly 1² NK-1⁺/11 shared other properties of basophilic leukocytes. Basophils are circulating granulocytes that participate in IgE antibody-mediated and T lymphocyte-dependent

Table 1 Number of Fc_εR on cloned mouse cells with natural killer function.

Cell	Fc _ε R × 10 ⁻⁴ per cell	No. of determinations
Cl. Ly 1 ² NK-1 ⁺ /11	7.9 ± 1.1	4
Cl. MC/9	9.6 ± 1.4	3
Normal peritoneal mast cells	23.0 ± 5.9	12
Cl. Ly 1 ² /9	0	2
YAC-1	0	2

The cell lines tested were Cl. Ly 1² NK-1⁺/11 (ref. 8), cloned (Cl. MC/9)²⁸ or normal peritoneal mouse mast cells, cloned inducer T lymphocytes (Cl. Ly 1² /9)³⁴ and the YAC-1 lymphoma⁸. Normal BALB/c mouse peritoneal mast cells were purified to >88% on a metrizamide gradient³⁵. The other cells were maintained as previously described. Fc_εR were enumerated as described elsewhere^{35,36} using ¹²⁵I-labelled, affinity-purified mouse monoclonal IgE antibody from hybridoma H 1 DNP-ε-26 (ref. 37). Results are expressed as the mean or mean ± s.e.

inflammatory processes²⁰. They express surface receptors (Fc_εR) that bind IgE with high affinity^{13,14} as do mast cells¹⁵. Fc_εR have also been demonstrated on certain lymphocytes, monocytes, macrophages and eosinophils²¹⁻²⁶. However, the receptors of basophils and mast cells differ from those of other cells^{21,22} by their higher affinity for IgE with low dissociation rate. Analysis of Cl. Ly 1⁻2⁻NK-1⁺/11 by direct binding of ¹²⁵I-labelled monoclonal mouse IgE demonstrated that it expressed high-affinity Fc_εR (Tables 1, 2). The binding of ¹²⁵I-IgE was specific: it was inhibited by unlabelled mouse IgE but neither by normal mouse IgG nor by rabbit IgG, even in 133-fold molar excess. The equilibrium constant at 37 °C (Table

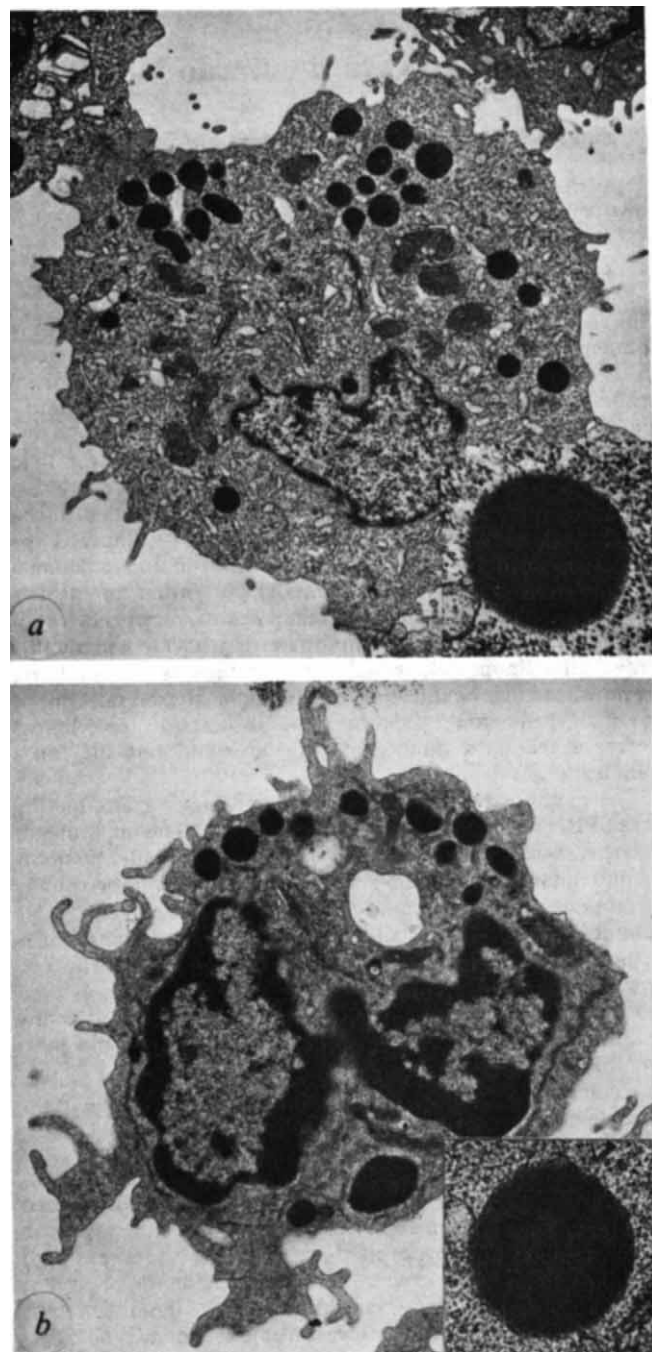


Fig. 1 *a*, Typical ultrastructure of Cl. Ly 1⁻2⁻NK-1⁺/11. Note the prominent cytoplasmic granules containing finely granular, homogeneous, electron-dense matrix. This cell also has a lobular nucleus with dispersed chromatin, an active Golgi area and abundant rough endoplasmic reticulum, features of immaturity. $\times 5,440$. Inset of granule, $\times 18,360$. *b*, Mature basophil from the bone marrow of a 6-week-old C57BL/6 mouse. The cytoplasmic granules are identical to those of Cl. Ly 1⁻2⁻NK-1⁺/11. $\times 10,880$. Inset of granule, $\times 29,900$.

2, Fig. 2) was similar to those of normal mouse or rat peritoneal mast cells, cloned mouse mast cells and the rat basophilic leukaemia cell line; it differs from the low-affinity Fc_εR of other cells by two orders of magnitude (Table 2).

Until recently, mice were considered devoid of basophils^{20,27}. We have now identified these cells with certainty by electron microscopy but they are very rare, accounting for $\leq 0.3\%$ of nucleated cells in C57BL/6 bone marrow²⁷. Their surface glycoprotein phenotype, biochemistry and function are unknown. In contrast to mouse basophils, mouse mast cells can be purified in large numbers and have been studied in detail. Like Cl. Ly 1⁻2⁻NK-1⁺/11, cloned mouse mast cells²⁸ express the Ly 5 glycoprotein and the Qat 4 and Qat 5 surface antigens and lack the Lyt 1 and Lyt 2 glycoproteins. Unlike Cl. Ly 1⁻2⁻NK-1⁺/11, cloned mast cells lack the Thy 1 and NK-1 surface antigens and do not mediate NK lysis²⁹. In addition, they differ from Cl. Ly 1⁻2⁻NK-1⁺/11 by ultrastructure and in their ability to synthesize and store histamine. Cl. Ly 1⁻2⁻NK-1⁺/11 therefore appears to represent a lineage distinct from mast cells, but more similar to basophils than previously realized. However, unlike the basophils of many species, Cl. Ly 1⁻2⁻NK-1⁺/11 does not synthesize or store histamine²⁸. Until methods are developed to isolate mouse basophils in large numbers, we will not know whether Cl. Ly 1⁻2⁻NK-1⁺/11 is in the basophil lineage or merely expresses certain remarkable basophil-like features. Regardless of its ontological relationship to basophils, the presence of high-affinity Fc_εR on Cl. Ly 1⁻2⁻NK-1⁺/11 suggests that the function of some cells with NK activity may be influenced by IgE antibody and specific antigen. This possibility is now being tested.

NK lysis has been associated with a variety of different cells in the myeloid or lymphoid series⁹⁻¹². In addition to Cl. Ly 1⁻2⁻NK-1⁺/11, at least two populations of cells mediating NK activity have been described in the mouse, one resembling small lymphocytes³⁰, the other resembling promonocytes³¹.

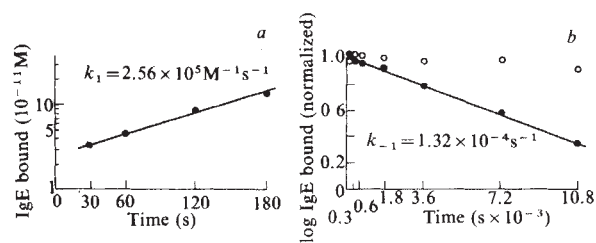


Fig. 2 *a*, Forward rate constant (k_1) of IgE binding to Cl. Ly 1⁻2⁻NK-1⁺/11 cells. Prewarmed cells ($1.4 \times 10^6 \text{ ml}^{-1}$) were incubated with $3 \mu\text{g ml}^{-1}$ of ¹²⁵I-labelled mouse monoclonal IgE at 37 °C in Dulbecco's modified Eagle's medium containing 10% fetal calf serum and 0.01 M EDTA with constant shaking. The binding reaction was stopped by adding a 133-fold excess of unlabelled IgE at the time indicated. Cells in control tubes were incubated with a 133-fold excess of unlabelled IgE before addition of ¹²⁵I-labelled IgE. Nonspecific binding of radioactivity to control cells was subtracted from experimental tubes to determine specific binding. Each point is the mean of duplicate measurements. The number of IgE receptors per cell was determined separately by incubating the cells with $10 \mu\text{g ml}^{-1}$ of ¹²⁵I-labelled IgE for 90 min. In this experiment, the average number of receptors was 9.26×10^4 per cell. *b*, Dissociation rate (k_{-1}) of IgE bound to Cl. Ly 1⁻2⁻NK-1⁺/11 cells. Cells ($3.9 \times 10^6 \text{ ml}^{-1}$) were incubated with $3 \mu\text{g ml}^{-1}$ of ¹²⁵I-labelled mouse monoclonal IgE at 37 °C for 1 h in Dulbecco's modified Eagle's medium with 10% fetal calf serum and 0.01 M EDTA. Cells were then divided into two tubes, centrifuged and resuspended in fresh medium. A 133-fold molar excess of unlabelled IgE (●) or an equal volume of culture medium (○) was added to each tube at t_0 . The cells were incubated at 37 °C with constant slow rotation and cell-bound ¹²⁵I-labelled IgE was determined at the times indicated. Each point is the mean of duplicate measurements. Data have been normalized to compensate for the slight difference in cell-bound IgE concentration in experimental (●) and control (○) preparations at t_0 . Cell viability at the start and end of the experiment was 99% and 89%.

Table 2 Binding characteristics of IgE to Fc_εR on cloned mouse cells with natural killer function.

Cell	k_1 (M ⁻¹ s ⁻¹ × 10 ⁵)	k_{-1} (s ⁻¹ × 10 ⁻⁴)	K_A (M ⁻¹ × 10 ⁹)
Cl. Ly 1 ⁻ 2 ⁻ NK-1 ⁺ /11	2.56	1.32	1.94
Cl. MC/9	0.82	0.79	1.04
Mouse peritoneal mast cells ³⁵	1.94	1.11	1.75
Rat peritoneal mast cells ³⁵	1.65	0.21	7.86
Rat basophilic leukaemia ³⁵	1.49	0.19	7.84
Rat basophilic leukaemia ³⁶	0.96	0.16	6

The cell lines tested were Cl. Ly 1⁻2⁻NK-1⁺/11, cloned mouse mast cells (Cl. MC/9)²⁸, normal mouse or rat peritoneal mast cells and the rat basophilic leukaemia cell line. All the values except those for the rat basophilic leukaemia cell line taken from ref. 36 were derived in the laboratory of T.I. using the same reagents. Quantitative determinations of association (k_1), dissociation (k_{-1}) and equilibrium (K_A) constants such as those shown require highly purified or homogeneous cell populations expressing high-affinity Fc_εR with low dissociation rates. In contrast, when these criteria cannot be met, K_A may be estimated by the Scatchard method or other indirect approaches. Estimated K_A values for human basophils are ~10⁹ (refs 13, 14). In contrast to the Fc_εR of basophils, mast cells and Cl. Ly 1⁻2⁻NK-1⁺/11, the Fc_εR of other cells have rapid dissociation rates ($t^{1/2} < 1$ min) with K_A of ~10⁷ (ref. 22).

Human peripheral blood leukocytes enriched for granulocyte precursors also express high levels of NK cell activity^{32,33}. We have shown that a cloned cell line mediating NK lysis *in vitro* expresses high affinity Fc_εR and contains cytoplasmic granules similar to those of basophils. This clone may be derived from a subset of cells in the granulocyte lineage, but the extent to which it is representative of other cells with NK function remains to be determined. Analysis of additional NK cells and the development of techniques to purify mouse basophils may elucidate the question of NK cell heterogeneity and their relationship to basophilic granulocytes.

We thank J. Goldin, R. Monahan, K. Pyne and A. Sterk for technical assistance. The work was supported by USPHS grants CA 28834, AI 16925, GM 07753, AI 12184, AI 13600, and AI 10060.

Received 19 January; accepted 7 May 1982.

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Acanthocytosis and cholesterol enrichment decrease lipid fluidity of only the outer human erythrocyte membrane leaflet

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The structure^{1–3} and functions^{4–10} of the human erythrocyte are influenced by the composition and organization of the membrane lipids. The outer (exofacial) and inner (endofacial) leaflets of the erythrocyte membrane differ in lipid composition^{11,12}, and recent studies using a group of membrane-impermeant pyrene fluorophores have demonstrated that the lipid fluidity of the outer leaflet exceeds that of the inner¹³. Using one of these probes, pyrene butyryl hydrazide linked to the tetrasaccharide stachyose (SPBH)¹³, we have compared the lipid fluidity of the outer and inner leaflets in normal human erythrocytes treated experimentally to alter membrane cholesterol content¹⁴ and in acanthocytes, erythrocytes of altered morphology found in individuals with the genetic disorder abetalipoproteinaemia¹⁵. The results, reported here, demonstrate that hemileaflet fluidity can be altered selectively: acanthocytosis and experimental cholesterol enrichment decrease the lipid fluidity of the outer but not the inner hemileaflet.

The methods used to assess outer and inner leaflet fluidity with SPBH have been described previously¹³. The impermeant probe is inserted selectively into the outer hemileaflet by treatment of intact cells, and after washing the cells to remove excess fluorophore, the fluorescence anisotropy is estimated by steady-state fluorescence polarization. When prepared in this manner suspensions of intact erythrocytes (0.02–0.05% packed cell volume) and of haemoglobin-free ghost membranes derived from them yield almost identical fluorescence anisotropy values¹³, indicating that osmotic lysis and isolation of the ghost membranes do not produce any major change in lipid fluidity or significant redistribution of the probe from the outer to the inner leaflet. Treatment of leaky ghost membranes with SPBH labels both leaflets, but primarily the inner leaflet, due to greater uptake at the endofacial surface. As noted previously¹³, comparisons of erythrocyte-loaded (outer leaflet) and ghost-loaded (mainly inner leaflet) membrane preparations with respect to fluorescence anisotropy, excited-state lifetime and intensity of excimer fluorescence provide concordant evidence of greater lipid fluidity in the outer as compared with the inner leaflet.

Normal human erythrocytes were treated to modify the membrane cholesterol content, and the effects on hemileaflet fluidity were examined in six experiments summarized in Table 1. In five experiments cholesterol content was altered by incubation with sonicated dispersions of cholesterol and dipalmitoyl phosphatidylcholine, as described by Cooper *et al.*¹⁶; molar ratios of cholesterol/dipalmitoyl phosphatidylcholine in the dispersions were either 0 (cholesterol depletion), 1 (controls) or 2–3 (cholesterol enrichment). In an additional experiment cholesterol content was modified by incubating the cells in an

Table 1 Fluorescence anisotropy of SPBH in erythrocyte-loaded and ghost-loaded membranes of cholesterol-modified cells

Expt	Cholesterol/phospholipid molar ratio			SPBH fluorescence anisotropy parameter $[(r_0/r) - 1]^{-1}$					
				Erythrocyte-loaded			Ghost-loaded		
	Cholesterol-enriched	Control	Cholesterol-depleted	Cholesterol-enriched	Control	Cholesterol-depleted	Cholesterol-enriched	Control	Cholesterol-depleted
1	1.20	0.87	0.55	0.26	0.21	0.11	0.26	0.26	0.23
2	1.56	0.76	0.39	0.35	0.20	0.19	0.31	0.32	0.24
3	1.69	0.90	0.45	0.22	0.16	0.17	0.33	0.33	0.26
4	1.25	0.71	0.46	0.27	0.20	0.15	0.34	0.38	0.30
5	1.53	0.86	0.67	0.34	0.22	0.21	0.46	0.49	0.34
6	1.33	0.84	0.42	0.24	0.19	0.17			

Intact human erythrocytes (20% suspension) or leaky ghost membranes (1.25–1.5 mg protein ml⁻¹) prepared from them were incubated with various concentrations of SPBH (intact cells, 75–225 µM; leaky ghosts, 5–20 µM) for 30 min at 37 °C. Membranes and cells were washed to remove unincorporated probe and the cells were then lysed osmotically and membranes prepared¹³. All membrane suspensions were adjusted to a protein concentration of 0.15–0.20 mg ml⁻¹ and steady-state fluorescence polarization estimated in an SLM polarization spectrofluorometer, with corrections for light scattering as previously described¹³. Total fluorescence intensities were similar in the erythrocyte- and ghost-loaded samples. In the range of probe concentrations achieved in these experiments, concentration-dependent depolarization was not observed¹³. Cholesterol modification was induced by incubation with sonicated dispersions of lipid¹⁶ in expts 2–6 and by treatment with an albumin-containing vehicle¹⁷ in expt 1. Values for the SPBH anisotropy parameter are means of triplicate determinations at 24 °C. The r_0 value is 0.119 (ref. 13). The fluorescence anisotropy (r) values (mean ± s.e.) for erythrocyte-loaded preparations are 0.020 ± 0.001 (controls), 0.026 ± 0.001 (cholesterol-enriched; $P < 0.005$ for difference from the controls) and 0.017 ± 0.001 (cholesterol-depleted; $P < 0.05$ for difference from the controls). Corresponding values for ghost-loaded preparations are 0.031 ± 0.002 (controls), 0.030 ± 0.002 (cholesterol-enriched; not significantly different from the controls) and 0.026 ± 0.002 (cholesterol-depleted; $P < 0.005$).

Table 2 Fluorescence polarization of SPBH in membranes of erythrocyte-loaded and ghost-loaded acanthocytes

Subject	Fluorescence anisotropy of SPBH		SPBH $[(r_0/r) - 1]^{-1}$		Sphingomyelin/lecithin ratio*
	Erythrocyte-loaded	Ghost-loaded	Erythrocyte-loaded	Ghost-loaded	
Patient M.S.	0.025	0.031	0.26	0.35	1.61
Normal A.C.	0.020	0.030	0.20	0.34	0.84
Patient M.S.	0.016	0.027	0.16	0.29	1.61
Normal P.S.	0.012	0.028	0.11	0.30	0.84
Patient A.M.V.	0.014	0.019	0.13	0.19	1.45
Normal P.Y.	0.011	0.019	0.10	0.19	0.84

Experiments were performed as described for the studies of cholesterol-modified erythrocytes. SPBH fluorescence anisotropy values are means of triplicate determinations at 24 °C. Those of erythrocyte-loaded preparations differ significantly ($P < 0.02$) in patient versus normal samples (paired t -test).

* Values reported by Cooper *et al.*²¹ for these patients; the normal value is a population mean²¹. Cholesterol content (µg per 10⁸ cells) of the patients' membranes were reported²¹ as 17.4 (M.S.) and 16.5 (A.M.V.) (mean normal value 14.6); cholesterol/phospholipid molar ratios were 1.04 (M.S.) and 1.02 (A.M.V.) (mean normal value 0.95).

isotonic vehicle containing either no lipid (control), cholesterol (enrichment) or egg lecithin (depletion), as described by Shinitzky *et al.*¹⁷. Changes in membrane lipid were monitored directly by chemical estimations of cholesterol¹⁸ and total phospholipid¹⁹ or indirectly by determination of the fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH)²⁰. The DPH fluorescence anisotropy parameter, $[(r_0/r) - 1]^{-1}$, where r is the observed fluorescence anisotropy and r_0 the maximal limiting anisotropy of the probe, varies directly with the cholesterol/phospholipid molar ratio (C/PL) of the membrane^{6,10} and is inversely related to the lipid fluidity. In the experiments summarized in Table 1, the DPH $[(r_0/r) - 1]^{-1}$ values at 24 °C were increased by $40 \pm 13\%$ (mean ± s.e.; $P < 0.001$) and decreased by $21 \pm 7\%$ ($P < 0.001$) in cholesterol-enriched and -depleted membranes, respectively, compared with the controls. Correspondingly, the C/PL ratios of the control, enriched and depleted membranes, respectively, were 0.82 ± 0.07 , 1.43 ± 0.19 and 0.49 ± 0.10 .

The results in Table 1 demonstrate that cholesterol enrichment increased the anisotropy parameter of the impermeant SPBH only in the erythrocyte-loaded (outer leaflet) preparations ($P < 0.005$); no significant effect was detected in the ghost-loaded suspensions. In control erythrocytes (C/PL = 0.82) the SPBH anisotropy parameter was 1.8 ± 0.4 times greater in ghost-loaded than erythrocyte-loaded membranes, in agreement with previous results¹³ and indicative of the more fluid outer leaflet. Cholesterol enrichment decreased this anisotropy parameter ratio to 1.2 ± 0.3 ($P < 0.05$) and thus diminished the asymmetry of leaflet fluidity characteristic of control cells.

The data in Table 1 also show that cholesterol depletion decreased the SPBH anisotropy parameter by $21.9 \pm 3.1\%$ ($P < 0.01$) in ghost-loaded preparations, with a less consistent decrease of $14.4 \pm 8.0\%$ ($P < 0.05$) in the erythrocyte-loaded

preparations. These observations demonstrate that SPBH can, in fact, 'sense' changes in lipid composition of ghost-loaded (mainly inner leaflet) membrane suspensions.

Erythrocytes obtained from two patients with abetalipoproteinaemia and from normal controls were examined with DPH and SPBH. The DPH fluorescence anisotropy parameter (24 °C) of the acanthocyte ghost membranes was $15 \pm 2\%$ greater than that of the normal controls, in agreement with previous observations^{21,22}. Further, as shown in Table 2, the fluorescence anisotropy parameter of SPBH in the acanthocyte preparations also exceeded that of the controls, but the increment of $37 \pm 5\%$ was observed only in the erythrocyte-loaded (outer leaflet) preparations. In the corresponding ghost-loaded suspensions the values for the normal and acanthocyte membranes were essentially identical.

That acanthocytosis decreases the lipid fluidity of the outer leaflet alone is explicable in terms of known changes in lipid composition of these abnormal membranes. Cooper *et al.*²¹ have reported that the sphingomyelin/lecithin molar ratio is increased in the acanthocyte membranes obtained from the patients in Table 2. Sphingomyelin is located primarily in the outer leaflet of the erythrocyte membrane^{11,12} and has been shown to decrease the fluidity of lipid bilayers²¹.

The effects on hemileaflet fluidity of alterations in membrane cholesterol are less readily explained, inasmuch as the hemileaflet distribution of the sterol has not been established. One simple interpretation of our results is that exogenous cholesterol is incorporated preferentially into the outer, more fluid leaflet. Cholesterol is removed, on the other hand, from both leaflets, but more readily from the inner, less fluid leaflet.

This research was supported by NIH grants HL16851, AM21238 and GM07367. We thank Dr H. J. Kayden for providing the blood samples of the patients with abetalipoproteinaemia.

Received 11 January; accepted 10 May 1982.

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Slow inward calcium currents have no obvious role in muscle excitation-contraction coupling

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It has been proposed¹ that an influx of calcium ions into twitch muscle fibres during an action potential might initiate contraction. However, when external Ca^{2+} is lowered to 10^{-8} M with EGTA, the fibres can produce normal twitches for many minutes^{2,3}. Nevertheless, a clear Ca^{2+} influx during contraction has been demonstrated^{4,5}, and it has been found that phasic skeletal muscle has an inward calcium current (I_{Ca})^{6,7} which can give rise to calcium spikes⁸. In certain conditions, a reduction in external Ca^{2+} with 80–90 mM EGTA results in reversible blockade of excitation-contraction (e-c) coupling⁹, leading some authors to suggest^{7,9–11} that extracellular Ca^{2+} moved into the myoplasm due to I_{Ca} may be involved in the e-c coupling mechanism that triggers contraction. This proposition was further supported by the localization of I_{Ca} in the T-system, which circumvented the problem of the delay due to calcium diffusion from the surface membrane. We have now investigated whether I_{Ca} has a clear role in initiating or sustaining contractions in twitch muscle fibres. Our approach was to decrease or eliminate I_{Ca} with the calcium-blocking agent diltiazem (Herbesser) and to see how the twitch, tetanic and potassium-contraction tensions were affected. We found that I_{Ca} could be decreased or cancelled with the calcium-blocking agent, but that the same concentration of the drug potentiated the twitch, tetanus and contractures. We conclude, therefore, that I_{Ca} has no role in e-c coupling. A preliminary report of these results has been presented elsewhere¹².

Two preparations were used: cut fibres for voltage-clamp studies, following the technique of Hille and Campbell¹³, and intact fresh fibres for contractility studies. For the first type, the fibre was voltage clamped with a holding potential of -100 mV, and test pulses were delivered. Test solutions were applied to the fibre exterior, added in the centre right pool bathed with normal Ringer. Membrane current recordings were

taken from the controlled membrane. For the contractility experiments tension was recorded from the isolated fibre with an RCA 5734 electromechanical transducer¹⁴. Diltiazem¹⁵, which blocks calcium currents in the heart¹⁶, was used in both types of experiment. All experiments were performed at room temperature (20 – 24 °C).

Our first aim was to see if the characteristics of I_{Ca} obtained in the present work were similar to those reported previously^{6,7,11}. (These experiments were performed with the voltage-clamp system.) Figure 1a shows I_{Ca} during a depolarizing pulse from -100 mV to -27 mV. This inward current first appears at -45 to -50 mV. It becomes faster, and increases in magnitude with the test voltage pulses used to drive the membrane potential. Depolarizations beyond -22 mV result in still faster kinetics but the current magnitude decreases, as shown by the current-voltage (I - V) relationship for the slow inward current (Fig. 1d). The Ca^{2+} dependence of these inward currents is shown in Fig. 1b, in which the current approximately doubles when the extracellular Ca concentration ($[\text{Ca}^{2+}]_0$) is increased from 1.8 to 5 mM. The magnitude and time course of this inward current and its I - V relationship are similar to those reported by others^{6,7,11}. Furthermore, its clear $[\text{Ca}^{2+}]_0$ dependence led us to believe that we were observing the same inward calcium currents (I_{Ca}) as described by them.

We next examined the effect of calcium blockers on I_{Ca} using diltiazem^{15,16} as a I_{Ca} blocking agent. The effect of some I_{Ca} blockers in the heart depends not only on the concentration used but also on the frequency of depolarization and the membrane (holding) potential^{17,18}. In the present experiments the test pulses were given from -100 mV holding potential every 4–5 min.

Figure 1c shows the time course of the action of 3×10^{-7} M diltiazem on I_{Ca} when added to the extracellular solutions. After 8 min I_{Ca} had decreased by 13%, and it had practically disappeared after 16 min. As Fig. 1d shows, 5×10^{-8} M diltiazem reduced the magnitude of I_{Ca} to 60% of the control value (determined on another piece of the same fibre); this effect was reversible. The same was observed if the same piece of fibre was used with and without diltiazem.

The finding that the slow inward current had the characteristics of I_{Ca} (refs 6, 7) and that diltiazem could decrease or cancel this current prompted us to investigate the effect of the drug on intact isolated muscle fibres. A diltiazem-induced depression in tension development would presumably reflect the influence of the I_{Ca} on either triggering or controlling any of the steps in e-c coupling. As illustrated in Fig. 2a–d, diltiazem exerts no depressing effect on the tension development as it does on I_{Ca} . Furthermore, the drug potentiated the force developed by electrical stimulation either as single twitches produced every 2 s or as tetanic stimulation elicited 5 s after the single twitch, both of which are stimulating conditions known to produce a strong inhibition of I_{Ca} (refs 17, 18). The twitch tension in the presence of diltiazem increased up to 80% over the control, and the time course of the twitches was also slightly modified. Comparison of the single twitches illustrated in Fig. 2a–f shows that under the influence of diltiazem the relaxation time and the duration of the twitch were prolonged, as was the duration of the relaxation after the last electric shock of a tetanic stimulation, while the tetanic tension was always greater throughout the period of activation. The increment of force development tended to decrease as the stimulation rate was increased because force development approaches the maximum tetanic force which the muscle can produce with or without the drug. The increase in twitch tension appeared approximately 5 min after addition of the drug and declined with a half time of 10 min after its removal. Further experiments investigating the influence of the I_{Ca} blocker on contractures due to sustained potassium depolarizations (Fig. 2e, f) showed that in the presence of diltiazem the 30 mM $[\text{K}^+]_0$ contracture produced after four twitches (1 every 20 s) had a time course similar to the control, but the force development was 1.15 times greater.

Fig. 1 Influence of diltiazem on slow inward currents in isolated cut muscle fibres. A small bundle of muscle cells were bathed with a solution containing (in mM): K-glutamate, 120; MgCl₂, 2; EGTA, 1; Tris-maleate, 5; ATP, 3; pH adjusted to 7. One fibre was dissected from the bundle and a 2-mm piece transferred to the type of experimental chamber described in detail by Hille and Campbell¹³. The bathing solution in the chamber was the same as above plus tetrodotoxin (1×10^{-6} g ml⁻¹), with Vaseline used to make the gaps; the gap in one of the pools (A, following the Hille and Campbell nomenclature) was ~ 250 μ m while the other pool (B) had a 350- μ m gap. Before the experiment was started, the capacitance of the membrane was measured with current pulses (control system in current mode). The fibre was discarded if the time course of the current pulses indicated a low capacitance, or the electronic decay in the voltage pulse was abnormal. *a*, Record of membrane current during a +73 mV command pulse from -100 mV (holding potential) to -27 mV. *b*, Effect of changing the external calcium concentration (as indicated to the right of each record) on the slow inward current due to a +68 mV command pulse. *c*, Time course of the effect of diltiazem on the slow inward current due to a +68 mV command pulse. 'c' is the control; at the right is shown the time at which each record was taken after the beginning of the exposure to 3×10^{-7} M of the drug. The calibrations are the same for the records *a*-*c*. *d*, The relationship between membrane current and voltage during different depolarization command pulses. $\circ$, Control; $\bullet$, after the fibre had been exposed to 5×10^{-8} M diltiazem for 30 min. Each set of records is from different fibres.

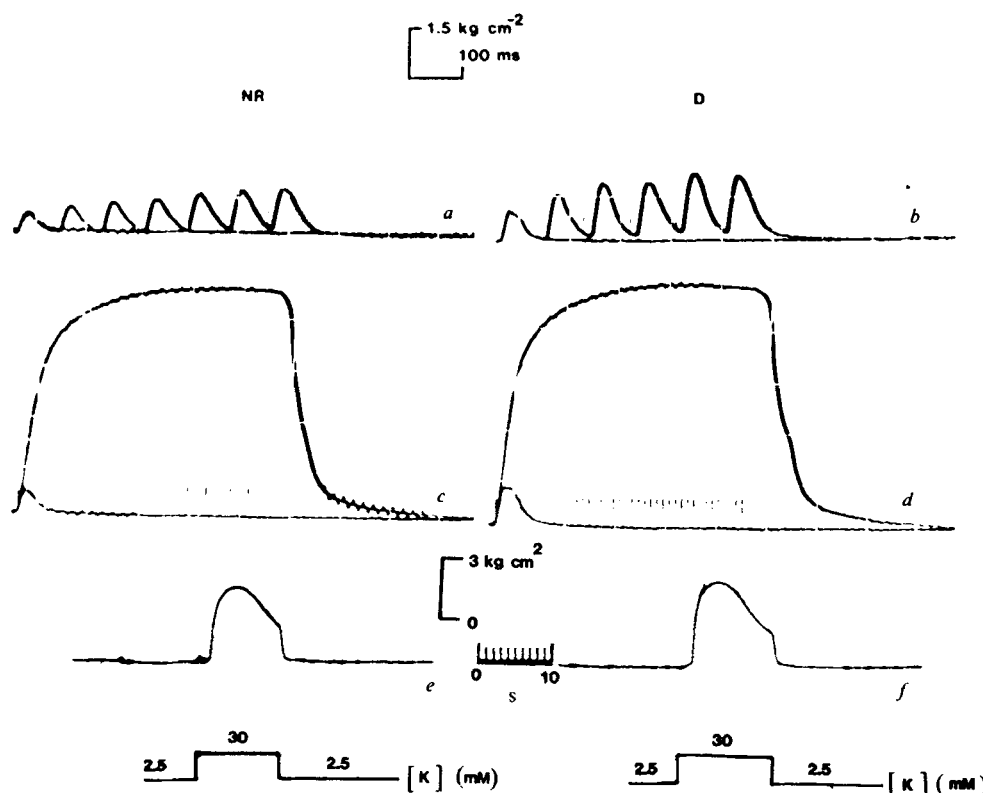
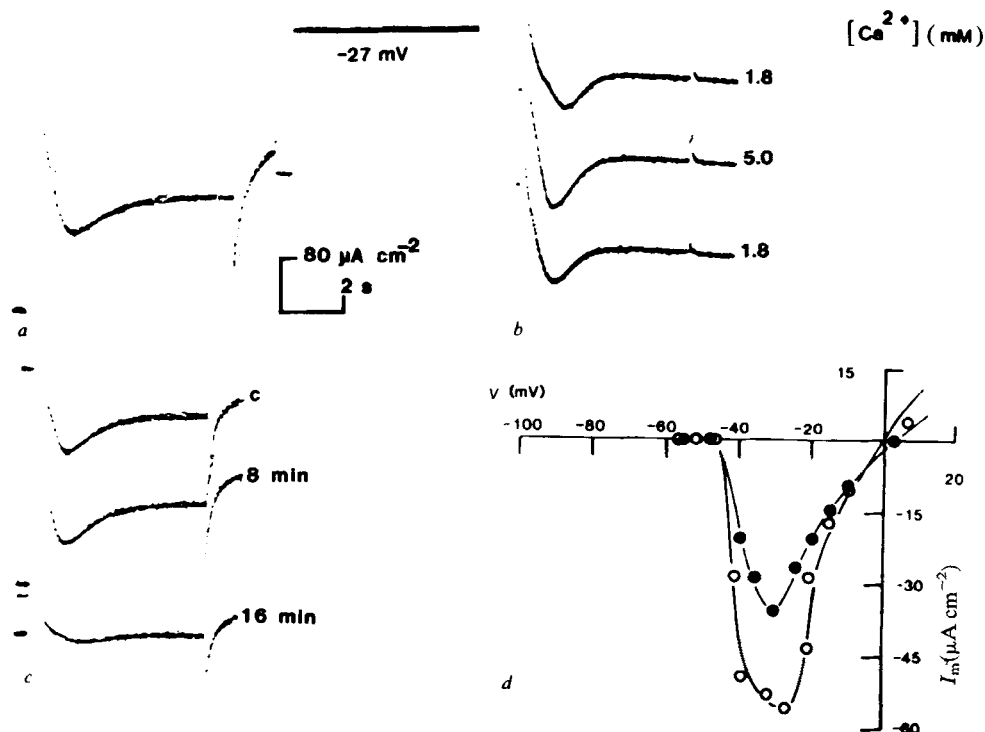


Fig. 2 Influence of diltiazem on force development of isolated single muscle fibres during electric stimulations and potassium depolarizations. The muscle fibre was isolated and mounted in a chamber described previously¹², where solutions could be changed easily and rapidly. NR, sample records taken from experiments performed in normal Ringer's; D, records taken from experiments performed in a Ringer's solution to which diltiazem had been added. Force development due to 10 (*a*, *b*) and 80 (*c*, *d*) Hz electrical stimulation. *b* Was taken after 15 min of exposure of the muscle cell to diltiazem (1×10^{-6} M). *d* Was taken 16 min after *b*. Between records *a* and *c* and *d*, some intermediate stimulation frequencies were tested. Except for the magnitude of the tetanic tension developed at these other frequencies, the results were similar to those described in the text, and shown here. *e*, *f* Are 30 mM potassium contractures after the fibre had been poisoned with tetrodotoxin (3×10^{-6} M); *e* is the control, *f* was obtained 23 min after the cell had been bathed with 1×10^{-6} M diltiazem. Top calibrations are for records *a*-*d*; bottom calibrations are for records *e* and *f*. Records *a*-*d* were from one muscle fibre, and records *e* and *f* from another fibre.

Our results show that diltiazem can decrease or abolish the slow inward currents which are probably due to Ca^{2+} without affecting significantly tension development due to brief electrical stimulation or sustained potassium depolarizations. On the contrary, the drug acts like a potentiator. From this, we conclude that I_{Ca} has no role in the e-c coupling mechanism that triggers contraction during normal electrical stimulation or steady potassium depolarization. It is unlikely that the diltiazem-induced inhibition of I_{Ca} did not produce twitch inhibition but rather that its potentiating effect simply overcame the inhibitory effect because twitch potentiation was still present with 3×10^{-6} M diltiazem, whereas I_{Ca} was almost abolished with 1×10^{-7} M (Fig. 1c). The present results are in agreement with previous results showing that external Ca^{2+} is not necessary to trigger contraction^{2,3}. A similar conclusion, based on different preliminary experiments, was suggested by McCleskey and Almers¹⁹. The mechanism by which diltiazem produces mechanical potentiation will require further analysis.

We thank Dr C. Armstrong for much helpful discussion and Drs Armstrong, A. P. Somlyo and G. McClellan for help in preparation of the manuscript. This work was supported by grants from NIH, 1 R01 NS 17048-01, Muscular Dystrophy Associations of America, Inc., and grant SEP 80-04 164 80 272, awarded to Dr Roberto Valle. M.C.G. was a trainee of CONACYT (Mexico). Diltiazem was supplied by Marion Laboratories.

Received 5 March; accepted 12 May 1982.

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Fetal myosin heavy chains in regenerating muscle

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There are several lines of evidence for the existence of a distinct class of myosins in developing muscle¹⁻¹⁰. Using various biochemical and immunological approaches, Whalen *et al.*⁹ recently suggested that two myosin heavy chain isozymes appear sequentially in rat muscle development, preceding the definitive adult myosins. It is unknown whether these myosins are present only in developing fast muscles or whether they also occur in developing slow muscles. Pyrophosphate gel electrophoresis studies have suggested that fast-twitch and slow-twitch muscles synthesize the same fetal myosin isozymes early in development⁶. Immunocytochemical studies with antibodies directed against adult fast and slow myosins show differences in myosin composition between fetal muscle fibres¹¹ but interpretation of these findings is complicated by cross-reactions of these antibodies with fetal isomyosins⁹. We have used a more direct

immunocytochemical approach to identify the myosin types present in developing muscle fibres. An antibody specific for bovine fetal myosin and cross-reactive with rat fetal myosin has been prepared. We report here that the fetal myosin heavy chains recognized by this antibody show a heterogeneous fibre distribution in fetal and neonatal rat muscle, disappear progressively during postnatal development and are transiently expressed in regenerating muscle.

Myosin was isolated from trunk and leg muscles of a 15-week-old bovine fetus as described previously¹². Antibodies to this myosin preparation were raised in rabbits and specific IgGs were separated by affinity chromatography^{12,13}. These antibodies intensely stained bovine and rat fetal muscle by indirect immunofluorescence but showed significant cross-reactivity with adult fast muscle fibres. Cross-reactive antibodies were eliminated by absorption with an ethanol-diethyl ether powder of myofibrillar proteins extracted from hind leg muscles of adult rat (S. Sartore, in preparation). Absorbed anti-bovine fetal myosin (anti-BFM), which was used for subsequent immunofluorescence studies, gave a single precipitin line on

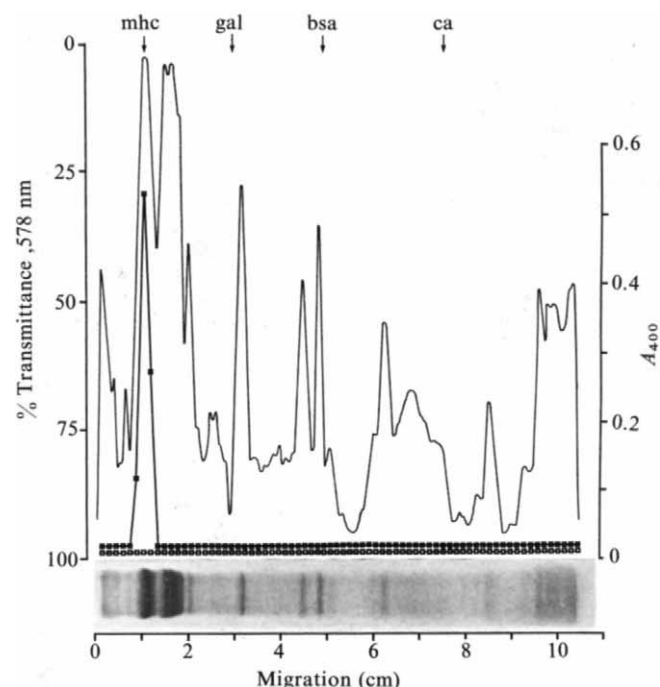


Fig. 1 Specificity of anti-BFM antibodies determined by enzyme immunoassay combined with SDS-gel electrophoresis. A crude myosin extract was isolated from leg muscles of 20 day-old rat fetuses. Pepstatin A ($200 \mu\text{g l}^{-1}$) and phenylmethylsulphonyl fluoride (0.1 mM) were present in all solutions. The muscle was homogenized in $50 \text{ mM KCl pH } 7.0$, washed three times in the same solution and extracted with $10 \text{ volumes of } 0.3 \text{ M KCl, } 0.15 \text{ M K-phosphate pH } 6.6$, containing $0.1 \text{ mM dithiothreitol (DTT)}$ and 5 mM Mg-ATP , for 30 min . After centrifugation, the supernatant was dialysed against $50 \text{ mM KCl, } 10 \text{ mM Tris-HCl pH } 7.2$, with 0.1 mM DTT . The resulting precipitate was heat-treated with SDS and 2-mercaptoethanol and run on a 10% SDS-polyacrylamide slab gel¹⁴. One lane was stained with Coomassie blue (lower panel) and scanned at 578 nm (upper trace), while two parallel lanes were sliced into $\sim 1.5 \text{ mm}$ strips. The slices were placed in $0.1 \text{ M Na-carbonate buffer pH } 9.6$, at 37°C for 24 h (to allow diffusion of protein from the slices), then assayed with anti-BFM (■) or preimmune rabbit IgG (□). Concentration of antibodies used was $0.9 \mu\text{g ml}^{-1}$. The enzyme immunoassay was carried out as described in ref. 15 with the modifications described in refs 16 and 17. Goat anti-rabbit IgG conjugated with alkaline phosphatase was used as second antibody with p -nitrophenylphosphate as the substrate. The enzyme activity was measured at 400 nm . SDS-polyacrylamide gel electrophoresis standards (BioRad) were used as molecular weight markers: mhc, myosin heavy chain; gal, β -galactosidase; bsa, bovine serum albumin; ca, carbonic anhydrase.

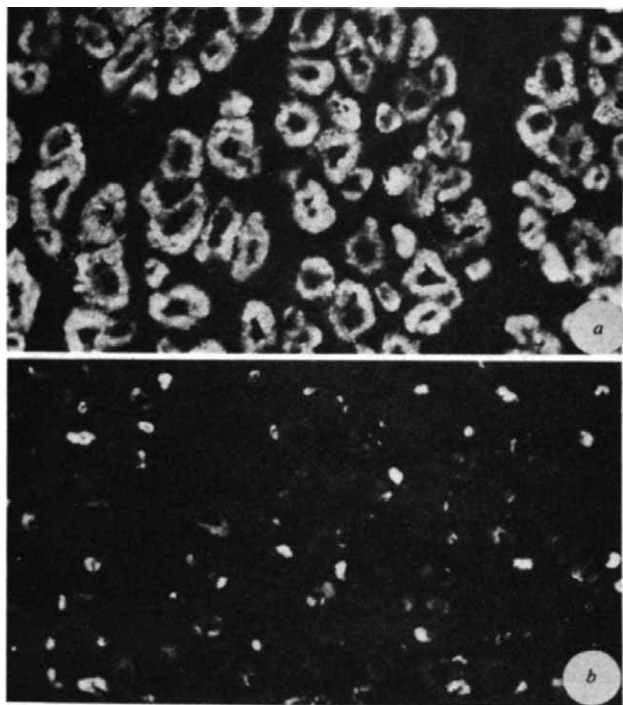


Fig. 2 Immunofluorescence staining with anti-BFM of tibialis anterior (a) and soleus (b) muscles from 19-day-old rat fetus. All muscle fibres are labelled in the tibialis. Two distinct populations of fibres can be identified in the soleus: large, primary generation myofibres unreactive with anti-BFM and small, secondary generation myofibres brightly labelled by anti-BFM. Indirect immunofluorescence assays were performed on transverse cryostat sections of the whole leg as described previously¹⁰.

double immunodiffusion Outcherlony plates against bovine and rat fetal myosin, stained selectively the A bands of the myofibrils in longitudinally sectioned bovine and rat fetal muscle, and reacted exclusively with myosin heavy chains when tested against a crude myosin preparation of rat fetal muscle by enzyme immunoassay combined with SDS-gel electrophoresis¹⁴⁻¹⁷ (Fig. 1). Immunofluorescence staining was completely abolished by preabsorption of anti-BFM with purified bovine fetal myosin heavy chains obtained from heavy chain bands cut out of 5% SDS slab gel electrophoretograms.

Most fibres in the fast-twitch tibialis anterior and extensor digitorum longus muscles were reactive with anti-BFM in 17-21-day rat fetuses (Fig. 2a). In contrast, primary generation myofibres¹¹ of the slow-twitch rat soleus muscle were not stained by anti-BFM (Fig. 2b). Differential fibre staining was also apparent in neonatal rat muscle. In serial sections treated with antisera against various adult myosins¹⁸ fibres showing reactions typical of fast fibres were labelled by anti-BFM, whereas fibres reacting as slow fibres were unlabelled. The timing of disappearance of anti-BFM staining varied among different subpopulations of fast fibres from ~3 weeks to 5-6 weeks after birth (S. Schiaffino, in preparation).

Similar differences were observed in the developing skeletal muscles of the rabbit. Muscle fibres in the fast-twitch longissimus dorsi muscle from a 2-day-old rabbit were stained by anti-BFM, whereas a large proportion of muscle fibres in the slow-twitch soleus, corresponding to primary generation myofibres, were unlabelled. This finding is especially interesting as the longissimus dorsi and soleus muscles of 2-day-old rabbits were found to display the same profile of fetal components when analysed by pyrophosphate gel electrophoresis⁵. Taken together, these results indicate that fast-twitch and slow-twitch muscles synthesize different types of fetal myosin isozymes early in development, and that anti-BFM reacts specifically with fetal

myosin heavy chains of the 'fast' type. Two distinct myosin heavy chain isozymes have been identified during fast muscle development⁹: an 'embryonic' isozyme is the predominant form in the 18-day rat fetus and a 'neonatal' isozyme is the predominant form in the first 2 weeks after birth. Absorption of anti-BFM with ethanol-ether powder of myofibrillar proteins extracted from the hind leg muscles of either 18-day rat fetuses or 2-week-old rats completely abolished immunoreactivity of both fetal and neonatal muscle. Thus it seems that anti-BFM antibodies are directed against determinants shared by 'embryonic' and 'neonatal' myosin heavy chains.

A cold injury was produced in adult rat muscles by applying dry ice to the surface of the tibialis anterior and soleus muscles of one leg. Injured and contralateral muscles were removed from day 1 to day 14 after the lesion. The morphological aspects of muscle degeneration and regeneration after cold injury were as described previously^{19,20}. Two days after the injury, muscle cells labelled by anti-BFM were identified in the tibialis and soleus first in the region intermediate between the superficial necrotic area and the underlying uninjured muscle. Some of the labelled cells had the shape and location typical of satellite elements. During the third and fourth days, several labelled myofibres were recognized at the site of the lesion (Fig. 3a). There was some variation in staining intensity among regenerating fibres, but no significant difference between regenerating fibres of tibialis and soleus was detected. Occasional surviving muscle fibres at the site of the injury were weakly reactive with anti-BFM. Newly formed myofibres subsequently increased in size, their reactivity with anti-BFM remaining almost

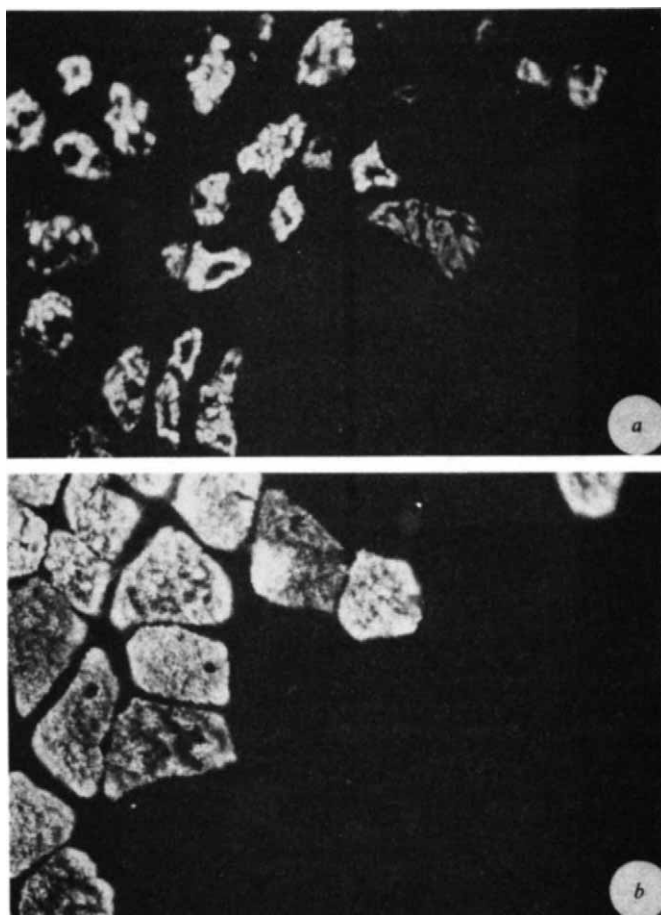


Fig. 3 a, Immunofluorescence staining of adult rat soleus muscle 3 days after local cold injury: newly formed fibres are brightly labelled by anti-BFM. Undamaged muscle fibres on the right are unlabelled. b, Immunofluorescence staining of adult rat tibialis anterior muscle 8 days after local cold injury. Regenerated fibres are still reactive with anti-BFM. Undamaged muscle fibres on the right are unlabelled.

unchanged 7–8 days after the injury (Fig. 3b). During the second week there was a progressive decrease in staining intensity and by 14 days most regenerated fibres could hardly be distinguished from the adjacent undamaged fibres.

Immunofluorescence studies were also performed on muscles denervated by sciatic nerve section 7 days before cold injury. The time course of reappearance of labelled regenerating fibres in the denervated tibialis and soleus was essentially similar during the first week after injury to that of normally innervated muscles. These results show that the myosin heavy chains recognized by anti-BFM can be expressed in both fast and slow regenerating muscles independently of neural influences. It remains to be established whether the absence of this fetal myosin from primary generation fibres of the soleus muscle is determined by an early influence of the nerve or whether other factors are involved.

Thus, we have described an antibody which reacts specifically with myosin heavy chains present in developing and regenerating muscle. On the basis of immunological evidence alone it is not possible to establish whether these myosin heavy chains are identical or simply antigenically similar. It is of interest that myosin types electrophoretically identical to those present in human fetal muscle have been identified in pathological conditions, such as Duchenne muscular dystrophy, when regenerative events are known to occur¹⁰. We have now found that fibres labelled by anti-BFM, which are absent in normal adult human muscle, are numerous in dystrophic muscle (in preparation).

This work was supported in part by a grant from the Muscular Dystrophy Association of America and the Legato Dino Ferrari.

Received 3 December 1981; accepted 13 May 1982.

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Conformation of terminal regions in proteins

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A carboxy-terminal helix has been observed in many proteins^{1–3}, suggesting that these helices confer an advantage, perhaps by providing protection against carboxypeptidase activity. To determine whether the conformational preferences of the amino- and carboxy-terminal regions are significantly different from each other and from the rest of the protein, we have analysed all proteins of known structure. We report here that the resulting distribution of the helical, β -strand and coil conformations is significantly different for the amino- and carboxy-terminals; the former preferentially adopts an extended β -strand while the latter is usually helical. The observed difference

derives from the α/β proteins⁴, in which the helix and strand alternate along the sequence, suggesting that the origin of this preference lies, not in protection against degradation, but in the special structural topology of α/β proteins and the $\beta\alpha$ unit.

For each protein from a sample of 70 non-homologous structures, the terminal 10 residues were classified as α or β , if the first secondary structure encountered from the terminus was a helix or sheet, respectively; if there was no secondary structure, it was classified as a coil. If most of the residues were not visible in the electron density map, its conformation was undefined. Turns were not included in this classification. The assignments for α and β were taken from hydrogen bonding diagrams, where available, and otherwise from the Cambridge Protein Data Bank⁵ or published author assignments. Figure 1 shows that the distribution of conformations for the N- and C-termini is significantly different; in a χ^2 analysis, the probability that this difference will occur by chance is less than 0.01. There are more than twice as many helices at the carboxy- than the amino-terminus and more strands at the N-terminus than the C-terminus in a ratio of $\sim 3:2$. The undefined conformations, which are usually considered flexible⁶, are predominantly (6:1) at the amino-terminus.

Table 1 Percentages of α -helix and β -sheet calculated for all residues and for the first ten (N_{10}) and last ten (C_{10}) residues in proteins

Protein (n)	% α			% β		
	All residues	N_{10}	C_{10}	All residues	N_{10}	C_{10}
All (54)	30	19 (0.02)	31 (0.9)	23	25 (0.9)	17 (0.2)
NOT α/β (37)	26	19 (0.2)	20 (0.2)	25	23 (0.9)	20 (0.3)
α/β (17)	38	18 (<0.001)	55 (<0.001)	20	30 (<0.02)	9 (<0.01)

The probabilities of observing the values by chance, calculated by χ^2 analysis, are given in parentheses.

To calculate the expected conformations of the termini, assuming that the secondary structure is randomly distributed along the sequence, the percentages of helix and sheet were evaluated for the entire polypeptide chain of each protein, and for the first and last 10 residues. These percentages were summed and averaged over the 54 non-homologous proteins for which data are available (see Table 1). Surprisingly, these data indicate that the amino-terminus has a low percentage of helical residues in contrast to the carboxy-terminus, which resembles the rest of the protein. On average there are more strands at the amino- and less at the carboxy-terminus than found for all residues. Calculations for the first and last 20 residues give percentages which are closer to the whole protein values.

The same analysis was performed separately for the α/β family of proteins⁴, in which helix and sheet alternate along

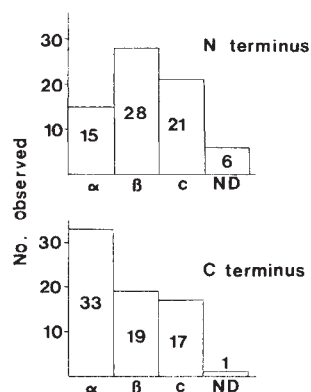


Fig. 1 Conformation of terminal regions (amino-terminus + 10 residues; carboxy-terminus - 10 residues) in 70 proteins. α , α -helix; β , β -sheet; c, coil; ND, not defined.

the sequence, and the NOT α/β proteins. Table 1 shows only slight deviations from expected values for the NOT α/β structures but large differences for the α/β proteins, in which there is a very strong preference for the N-terminal 10 residues to include a β -strand and not a helix, whereas the C-terminus is frequently helical, but rarely extended.

We examined the individual amino-terminal strands (28 proteins) and carboxy-terminal helices (33 proteins) to determine whether there were any common characteristics. The most striking difference is that the N-terminal strands are usually buried in the centre of the sheet, in contrast to the C-terminal helices which are predominantly exposed. The data for the amino-terminal strands show that there are less edge strands than expected by chance. A full analysis of the relative solvent accessibilities of the terminal regions is in progress.

Almost half of the proteins in the data base are extracellular and will probably be synthesized with an N-terminal signal peptide, which is later cleaved⁷. These signal sequences have high helical propensities and, if not removed from the protein, might be considered as N-terminal helical regions. However, this observation does not affect the results for the β/α proteins which are almost exclusively intracellular. In addition, the signal peptide is unlikely to influence the native structure of the secreted protein.

Thus, the amino- and carboxy-terminal regions have different conformational probabilities: the amino-terminus preferentially adopts an extended β -strand conformation while the carboxy-terminus is more usually helical. The observed

difference derives basically from the α/β proteins, suggesting that the origin of this preference lies not in protection against degradation but in the special structural topology of α/β proteins and the $\beta\alpha\beta$ unit. There are several possible explanations. Perhaps the simplest is that the $(\beta\alpha)_n$ structure is the basic unit in evolution, from which all α/β proteins are derived. In 44 $\beta_1\alpha\beta_2$ units, in which the parallel strands are adjacent in the sheet, there are more than twice as many alpha-carbon close contacts ($<6 \text{ \AA}$) between β_1 and the intervening helix α , than between β_2 and α . In addition, the secondary structure prediction using the method developed by Robson and co-workers⁸, is better for $\beta_1\alpha$ than for $\alpha\beta_2$ (W. R. Taylor, personal communication). This reinforces the concept of a basic $\beta\alpha$ structure, as opposed to a $\alpha\beta$ unit. Alternatively, from a sequential folding viewpoint, the most favourable structure for a $\beta\alpha\beta$ sequence is almost certainly parallel β -strands joined by an antiparallel helix. In contrast, an $\alpha\beta\alpha$ sequence would probably maximize the α - α contacts including helix dipole interactions⁹ in an antiparallel hairpin joined by an extended region. If folding proceeds from an amino-terminal nucleating core, this terminus would tend to be more hydrophobic and therefore incorporate more β -sheet than an exposed carboxy-terminus. A definitive explanation must await more experimental data on the folding pathways of α/β proteins.

We thank Professor Tom Blundell for his interest and encouragement and William Taylor for useful comments. J.M.T. holds a SERC Advanced Fellowship.

Received 31 March; accepted 13 May 1982.

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Phylogenetic origins and adaptive evolution of avian and mammalian haemoglobin genes

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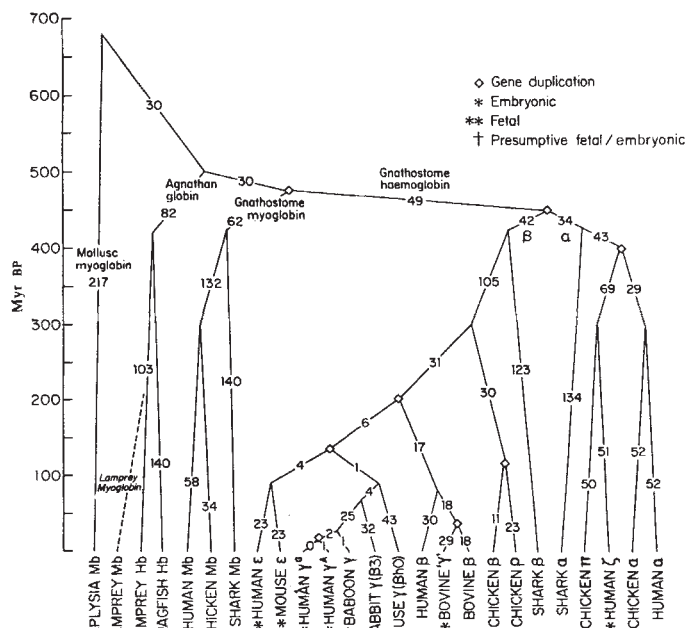
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Recent years have seen rapid growth in amino acid sequence data on globins and nucleotide sequence data on haemoglobin genes and pseudogenes, and cladistic analysis¹ of these data continues to reveal new facets of globin evolution. Our present findings demonstrate: (1) avian and mammalian embryonic α genes (π and ξ , respectively) had a monophyletic origin involving an α locus duplication about 400 Myr ago soon after the duplication which separated α and β genes; (2) much later in phylogeny, independent β -gene duplications produced the embryonic ρ locus of birds and embryonic ϵ and fetal γ loci of mammals. This parallels the earlier finding² that myoglobins evolved more than once from generalized globin ancestors. Here we support the view² that such globin evolution resulted from natural selection acting on mutations in duplicated genes. Thus, our evidence contradicts the neutralist view^{3,4} in which almost all amino acid substitutions in descent to extant globins evaded positive selection.

The facets of globin evolutionary history shown in Fig. 1 come from a genealogical reconstruction carried out on amino acid sequence data from 195 globin chains of 87 vertebrate and 19 non-vertebrate species. Figure 2 shows the genealogical reconstruction for 40 α - and β -haemoglobin genes and pseudogenes of 8 vertebrate species carried out on nucleotide sequence data representing coding regions of the expressed genes and corresponding regions of the unexpressed pseudogenes. Previous studies of globins¹ and other protein families, such as calcium binding proteins⁵, cytochromes *c* (ref. 6), α -lens crystallins⁷, carbonic anhydrase isozymes⁸ and ribonucleases⁹, indicate that genealogical reconstructions by the maximum parsimony procedure provide an effective way of extracting cladistic information from sequence data. The use of nucleotide sequences allows the numbers of nucleotide replacements which cause amino acid changes and those which do not (silent replacements) to be calculated for each branch of such a tree (see Fig. 2 and legend). The ratio of amino acid changing to silent substitutions R_{cs} value obtained from this calculation is one of three parameters that we used to evaluate the role of natural selection in globin evolution, the other two being rate of amino acid substitutions and distributions of substitutions in functionally different parts of the haemoglobin molecule. The changes in values of these parameters during different evolutionary periods provide evidence that a substantial proportion of amino acid substitutions were adaptive.

Myoglobin, β -haemoglobin and embryonic and adult α -haemoglobins of extant birds and mammals had their origins during early vertebrate phylogeny (Fig. 1). Assuming that the agnathan from gnathostome (jawed vertebrate) and shark from teleost-tetrapod globin-lineage splittings were congruent with the corresponding species-lineage splittings and that paleontological views on early vertebrates are not too inaccurate, rates of amino acid substitutions in these diverging early-vertebrate globin lineages were exceptionally fast, with rates as high as 110 NR% (nucleotide replacements per 100 codons per

Fig. 1 Representative lineages from the most parsimonious globin phylogenetic tree found for 195 globin amino acid sequences. The strategy for finding among the alternative trees examined the one with fewest genic changes was the same as that used in a previous cladistic analysis¹ of 172 globin sequences. The present analysis provides clear evidence on the phylogenetic origins of bird and mammal embryonic haemoglobin (Hb) sequences. For example, grouping the chicken embryonic α or π branch with the reptilian-avian adult α branch while grouping the human embryonic α or ζ branch with the mammalian adult α branch, rather than having the monophyletic origin of π and ζ sequences shown in the figure, adds 35 NRs to the tree. Conversely, having in the basal amniotes a monophyletic origin for mammalian embryonic β -like or ϵ chains and avian embryonic β -like or ρ chains, rather than the phylogenetically later independent origins shown in the figure, adds 30 NRs to the tree. The numbers of NRs shown on the lines of descent have been corrected for superimposed mutations by our augmentation algorithm⁶. The ordinate scale in Myr BP is based on palaeontological views, as previously used¹, concerning the ancestral separations of the organisms from which the 195 globins came. If the full tree were shown, it would be apparent why the gene duplications are placed in their particular positions on the ordinate scale. For example, in the full tree the embryonic α s (π and ζ) and teleost α s are joined together and diverge from the lineage to tetrapod adult α s. Thus, the duplication from which the embryonic and adult α loci emerged is deduced to have occurred just before the ancestral divergence of teleosts and tetrapods, that is at ~400 Myr BP. As another example, the divergence of the mammalian ϵ - γ line from the β line is placed at ~200 Myr BP because it is followed in the β line by the divergence of monotremes from therian mammals. Lamprey myoglobin (Mb) was not included in the actual computer reconstruction. However, the dashed line shows, from evidence reviewed elsewhere¹⁶, that this myoglobin is cladistically closer to lamprey haemoglobin than to any other globins. Sequences not previously catalogued^{1,16} are chicken α ¹⁷, human ζ ² (ref. 18), chicken ρ (ref. 19), mouse γ (β H0), (C. A. Hutchinson, S. J. Phillips, A. Hill, S. C. Hardies and M. H. Edgall, personal communication), rabbit γ (β 3)²⁰ and mouse ϵ ²¹. Computer simulations establish that our phylogenetic tree reconstruction algorithms capture such variables of the simulation as the frequencies of different types of nucleotide substitutions and the differential rates of evolutionary change in different tree regions and at different alignment positions (R. Holmquist, T. Conroy, J. C. and M.G., in preparation). The results also support the finding of an earlier study²² that the denser the phylogenetic tree the more accurate the maximum parsimony reconstruction. The density of sequences used for the present amino acid tree was sufficient to yield a reliable reconstruction.



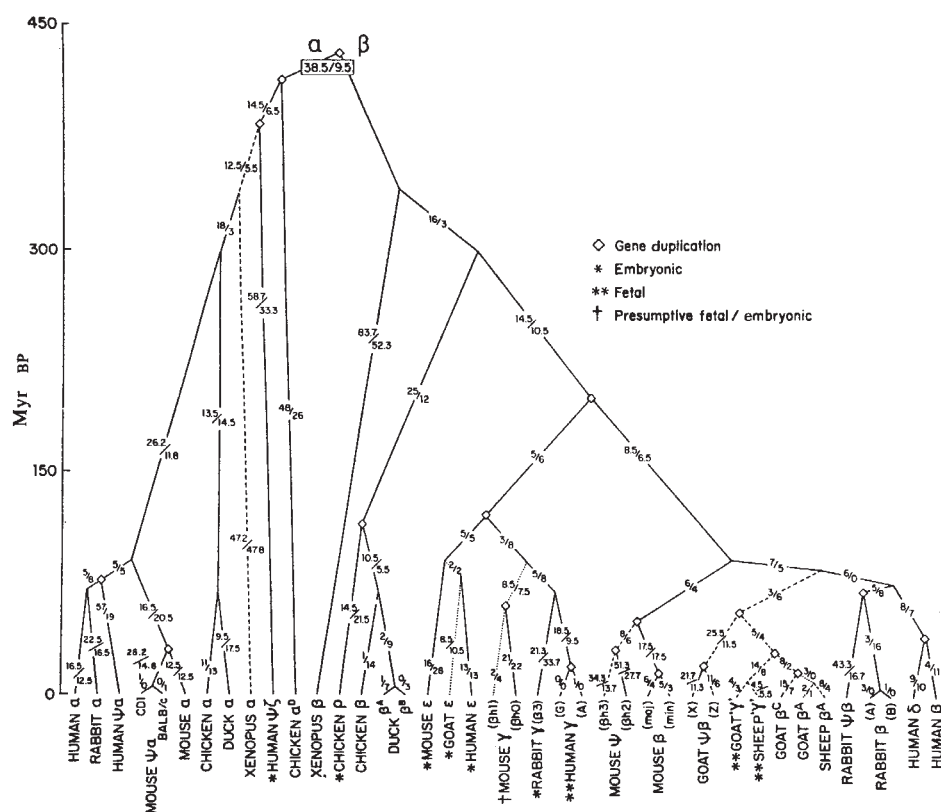
100 Myr). Between gnathostome and amniote (bird-reptile-mammal) ancestors (from ~425–300 Myr BP), rates were also fast, averaging 59 NR% in the four major globin lineages. However, later in vertebrate phylogeny from the amniote ancestor to present day birds and mammals, amino acid substitutions occurred at a much slower rate, averaging ~15 NR%. During the early period of fast globin evolution, the highest concentrations of amino acid substitutions were at residue positions which acquired new or altered functions. This was particularly true during the initial evolution of heterotetrameric haemoglobin following the gene duplication which produced separate α and β loci. The fastest rates seem to have occurred at those sites which became responsible for the subunit cooperativity permitting rapid unloading of oxygen in respiring tissues (Table 1). However, later in descent these cooperative sites, that is the $\alpha_1\beta_2$ contacts, Bohr effect sites and 2,3-diphosphoglycerate (DPG)-binding sites^{10,11}, along with the haem contacts, evolved at the slowest rate. It follows that natural selection, rather than random drift of selectively neutral mutations, was responsible for this nonrandom pattern of amino acid substitutions. A shift from higher to lower R_{cs} values during descent of the expressed α - and β -haemoglobin genes is possibly a further indication that positive selection for adaptive substitutions became stabilizing selection preserving perfected adaptations.

A standard for evaluating the R_{cs} values of lineages of expressed haemoglobin genes is provided by the R_{cs} values of lineages of unexpressed α - and β -haemoglobin pseudogenes. The various eutherian lineages of these α - and β -pseudogenes depicted in Fig. 2 have an average R_{cs} value of 2.2, somewhat less than that of randomly mutating codons ($R_{cs} = 3.0$). Previous calculations regarding the interval of time between the production by gene duplications of a proto-pseudogene lineage and its silencing suggest that the lower the proportion of amino acid changing to silent base replacements, the longer the interval before silencing¹². However, doubt must now be cast on the assumptions used in such calculations. Consider the two cladistically related goat ψ - β sequences: each possesses the same chain terminating mutation (thus indicating that they both originated from an ancestral sequence already silenced¹³), yet they have

an average R_{cs} value of only 1.9. Perhaps structural constraints operating on the chromosomal segments containing the expressed haemoglobin genes also operate on the unexpressed pseudogenes, preventing a strictly random accumulation of mutations. Moreover, the same nonrandom usage of bases in the third position of codons (for example, excesses of C-ending alanine, asparagine, glycine, threonine and tyrosine codons) found in α - and β -haemoglobin genes are found in the pseudogenes. Nevertheless, the high rate of evolution of eutherian haemoglobin pseudogenes indicates that they are under much less stringent selection for preservation of coding sequence structure than are expressed haemoglobin genes. In contrast to pseudogene lineages, expressed lineages frequently show R_{cs} values less than 1. This indicates that silent replacements are less constrained by stabilizing selection than are amino acid-changing replacements. However, when positive natural selection is transforming the sequence structure of a protein, elevated R_{cs} values in the range of pseudogenes and randomly mutating codons can be expected.

The amino acid-changing and silent base-replacement values recorded on the branches of the nucleotide sequence tree (Fig. 2), and the R_{cs} values calculated from them suggest that during differentiation of α - and β -haemoglobin loci there were many more amino acid-changing replacements than silent ones. A caveat is necessary because other methods¹⁴ imply that saturation of silent substitutions leads to high R_{cs} ratios between distantly related sequences. This suggests that the estimated R_{cs} values between amniote α and β ancestors, at the apex of the tree, do not reflect the actual R_{cs} values which occurred. We have tested, by simulation, that under the null hypothesis of a uniformly faster rate of silent to amino acid-changing substitutions, high R_{cs} values at the tree's apex would result from artefacts of our method. Results to be described elsewhere show that the apex R_{cs} values given in Table 1 and Fig. 2 are two to three times higher than those found in the simulations, thus indicating that during differentiation of α - and β -haemoglobin loci there was indeed a higher rate of amino acid-changing substitutions to silent. Moreover, the codons for sites which have the strongest functions in fully evolved

Fig. 2 Most parsimonious phylogenetic tree for 40 haemoglobin genes and pseudogenes. The nucleotide sequences were aligned to give 444 nucleotide positions; intervening sequences (introns), 3' and 5' non-coding DNA and insertions present only in the pseudogene sequences were not included in the alignment. The method used to construct the most parsimonious tree and calculate the branch lengths was that described by Goodman (ref. 1 refs therein) modified for use with nucleotide sequences. The numbers shown as a fraction on each branch or link are, in the numerator, the number of amino acid-changing base replacements and, in the denominator, the number of silent base replacements. Our computer algorithm carried out this calculation for each pair of ancestral and descendant-codons at each two adjacent nodes of the tree; if there is more than one mutation separating the two codons, all possible minimum pathways of getting through the genetic code from one codon to the other are examined, the number of amino acid-changing nucleotide replacements for each path are calculated, and the average is taken. For calculating the ratios of amino acid-changing to silent replacements (that is, R_{cs} values), the numbers recorded on the links for the two types of point mutations are originally observed or unaugmented NR values. Because relatively few contemporary sequences represent the long period of evolutionary time involved (from 450 Myr BP to the present), the numbers of both the amino acid-changing and silent NRs are almost certainly underestimated, especially at the apex of the tree and on the branches which start near the apex and descend to the present without intervening nodes. Nevertheless, the results of computer simulation tests of the accuracy of our maximum parsimony algorithm with regard to measuring the relative proportions of the two types of NRs (see text) suggest that the shift from higher R_{cs} values on earlier links of the tree to lower R_{cs} values within the amniotes is not an artefact of the maximum parsimony reconstruction but rather reflects a real evolutionary trend. The following complete sequences were used: human α^{23} , rabbit α^{24} , human $\psi\alpha^{25}$, mouse α^{26} , mouse $\psi\alpha$ strains CD1 and BALB/c^{27,28}, chicken α^{17} , duck α^{29} , human $\psi\zeta$ 1, (N. J. Proudfoot personal communication), chicken α^D (ref. 17), *Xenopus* $\beta^{30,31}$, chicken ρ^{19} , chicken β^{32} , duck β (ref. 33), duck β^B (ref. 34), mouse ϵ^{21} , human ϵ^{35} , mouse $\gamma(\beta h0)$ (C. A. Hutchinson, S. J. Phillips, A. Hill, S. C. Hardies and M. H. Edgall, personal communication), rabbit $\gamma(\beta 3)^{20}$, human γ and γ (ref. 36), mouse $\psi\beta h2$ (C. A. Hutchinson, S. J. Phillips, A. Hill, S. C. Hardies and M. H. Edgall, personal communication), mouse β -maj and β -min³⁷, goat $\psi\beta^*$ (ref. 38), rabbit $\psi\beta^{39}$, rabbit β and β alleles^{40,41}, human δ^{42} and human β^{43} . Also two sequences were used which had, due to extensive deletions, less than the full per cent of coding blocks: goat $\psi\beta^Z$ (76%)¹³ and mouse $\psi\beta h3$ (69%) (C. A. Hutchinson, S. J. Phillips, A. Hill, S. C. Hardies and M. H. Edgall, personal communication). In addition, the following partial sequences (portions of each sequence not having been determined by the time of our study) were used: *Xenopus* α (81%)⁴⁴, goat ϵ (35%)^{45,46}, mouse γ ($\beta h1$) (30%) (C. A. Hutchinson, S. J. Phillips, A. Hill, S. C. Hardies and M. H. Edgall, personal communication), goat γ (64%)^{45,46}, sheep γ (79%)⁴⁷, goat β (62%)^{45,46}, goat β (49%)^{45,46} and sheep β (72%)⁴⁷. Dotted lines: links on which the NR values represented very incomplete portions (in the range 30–49%) or the α or β alignment. Dashed lines: links on which the NR values represent more extensive portions (in the range 62–81%) of the α or β alignment. Note that the lineage to human $\psi\zeta$ 1 was for most of its history an expressed ζ lineage as the human $\psi\zeta$ 1 sequence shows only six changes (a deleted residue and five base differences due to four amino acid differences) from the expressed human ζ 2 sequence. Note also that certain of the indicated gene duplications ($\diamond$) might be gene conversions³⁶ as well as duplications. For example, the closer cladistic relationships of human γ and γ chains to one another than to baboon γ (Fig. 1) could be due to gene conversion in the hominoid lineage in as much as cercopithecoids like hominoids have two γ loci⁴⁸. Similarly, the closer cladistic relationship of bovid γ genes to bovid β genes than to γ genes in primates, lagomorphs and rodents could reflect a conversion of the older γ locus by the bovid β locus.



heterotetrameric haemoglobin (haem contacts and cooperative sites) clearly show, with average (av.) $R_{cs} > 5$, this higher rate of amino acid-changing replacements. Conversely, within the amniotes, not only did far fewer amino acid changing to silent replacements (R_{cs} often < 1) occur in the expressed α - and β -gene lines, but the codons for haemoglobin sites with the strongest functions accumulated the smallest proportion of amino acid-changing base replacements (av. $R_{cs} = 0.35$). Thus, we conclude that stringent selection in the amniotes was preserving the adaptive amino acid substitutions which had occurred during differentiation of α and β chains. This view on selection seems more logical than that of the neutralists^{3,4,12}, who believe that selection serves only as a conservative force. The extreme neutralist's view fails to explain the evolutionary origins of adaptive sequence structures—as though proteins were created with almost all of their adaptive features firmly in place.

Further evidence of positive selection of adaptive amino acid substitutions followed by stabilizing selection is provided by changes in rates of amino acid substitutions and in R_{cs} values

which occurred during shorter spans of evolutionary time and closer to the present than for the previous evidence presented. A good example of this is the pattern of changes on the lineage to catarrhine primate γ chains. From the primate-lagomorph ancestral node to catarrhine ancestral node (~70–25 Myr BP), the rate of amino acid-changing base replacements, as calculated from the amino acid sequence tree (Fig. 1), was six to seven times faster than from the catarrhine ancestral node to present (40 NR% compared with only ~6 NR%). During the earlier period of accelerated evolution in this primate γ line, three base replacements occurred at DPG-binding sites: valine at helical position NA1 mutated to glycine, and histidine at helical position H21 mutated through two amino acid-changing base replacements to serine. The loss of DPG-binding capacity brought about by these amino acid substitutions favoured the ability of fetal haemoglobin to capture oxygen from maternal haemoglobin. This may have helped make possible the relatively long period of fetal life coupled with extensive prenatal brain development distinguishing catarrhine primates from other eutherian mammals. That almost all the amino acid-

Table 1 Amino acid changing (C) and silent (S) nucleotide replacement rates: NR/codon position/100 Myr $\times 10$

Evolutionary lineages	Functional groups (no. of codon positions)										Total NRs		R_{ca}
	HC (23)		Coop (24)		$\alpha_1\beta_1$ (14)		IP (19)		Other (68)		All (148)		
	C	S	C	S	C	S	C	S	C	S	C	S	
<i>a</i> , α - β link	1.21	0.29	4.00	0.71	1.50	0.50	1.14	1.14	1.57	0.21	38.5	9.5	4.05
	HC (19)		Coop (16)		$\alpha_1\beta_1$ (15)		IP (21)		Other (70)		All (141)		
<i>b</i> , Stem to amniote α anc	0.42	0.00	0.50	0.50	3.92	1.08	2.00	2.00	3.67	0.83	45	15	3.00
<i>c</i> , Amniote α s	0.06	0.82	0.27	1.01	1.53	1.05	1.08	1.20	1.57	1.23	133.2	131.8	1.01
<i>d</i> , Bird α s	0.00	1.14	0.43	1.36	1.43	0.93	1.36	1.71	1.29	1.79	20.5	30.5	0.67
<i>e</i> , Eutherian α s	0.20	1.28	0.00	2.52	3.20	1.60	1.52	2.08	2.96	2.40	73	75	0.97
<i>f</i> , Eutherian $\psi\alpha$ s	3.45	1.82	5.36	1.55	4.82	1.18	4.73	1.73	6.55	2.91	86.2	34.8	2.48
	HC (23)		Coop (19)		$\alpha_1\beta_1$ (15)		IP (19)		Other (70)		All (146)		
<i>g</i> , Stem to amniote β anc	1.00	0.00	5.25	0.00	3.25	0.00	1.25	0.00	2.75	1.00	16	3	5.33
<i>h</i> , Amniote ρ - ϵ - γ - β s	0.53	0.99	0.29	1.00	1.64	1.60	1.13	1.19	1.52	1.52	276.9	317.1	0.87
<i>i</i> , Bird β s	0.00	1.73	0.00	0.73	0.87	0.87	0.00	1.40	0.20	1.80	4	33	0.12
<i>j</i> , Eutherian ϵ - γ - β s	0.86	1.49	0.34	1.59	2.33	2.41	1.64	1.41	2.20	2.14	186.9	211.1	0.89
<i>k</i> , Eutherian $\psi\beta$ s	3.82	2.88	7.29	2.88	8.71	5.00	4.18	1.24	6.35	3.00	149.8	73.2	2.05

Only lineages to complete sequences are included in the calculation of nucleotide replacement rates for the different functional groups of codon positions. The time scale for the rate calculations is that used in Fig. 2. Nodes representing gene duplications are placed on this time scale by extrapolation of the link-length NR values connecting such nodes to those which represent the species-splittings dated by palaeontological evidence. a, Time of α - β duplication is placed at 450 Myr BP, the first node in descent of the α stem (where chicken α^D joins other α s) at 420 Myr BP, and the *Xenopus*-amniote ancestral β node at 340 Myr BP; this yields a time of 140 Myr. b, Times of the connecting links from the first α node after the α - β duplication to the amniote α ancestral node, placed at 300 Myr BP, add up to 120 Myr. c, Times of the connecting links from the amniote α ancestral node to the extant complete sequences add up to 830 Myr. d, Times of the connecting links from the chicken-duck ancestral α node (placed at 70 Myr BP) to the present add up to 140 Myr. e, Times of connecting links from the eutherian α ancestral node (placed at 90 Myr) to the extant complete α sequences add up to 250 Myr. f, Times of the links from the ancestral nodes of these $\psi\alpha$ sequences with their nearest expressed α lineages to the $\psi\alpha$ sequences add up to 110 Myr. g, Time of the link from the *Xenopus*-amniote ancestral β node to amniote ancestral ρ - ϵ - γ - β node is 40 Myr. h, Times of the connecting links from the amniote ancestral ρ - ϵ - γ - β node to the present day ρ , ϵ , γ and β complete sequences add up to 1,630 Myr. i, Times of the connecting links from the duck-chicken ancestral β node to the present day duck and chicken β sequences add up to 150 Myr. j, Times of the connecting links from eutherian ϵ , eutherian γ and eutherian β ancestral nodes (each placed at 90 Myr BP) to present day complete ϵ , γ and β sequences, respectively, add up to 760 Myr. k, Times of the links from the ancestral nodes of these $\psi\beta$ sequences with their nearest expressed β lineages to the $\psi\beta$ sequences add up to 170 Myr. The functional groups of codon positions follow the scheme used for amino acid sequence data¹: HC haem contacts; Coop: the cooperative sites $\alpha_1\beta_2$ contact, Bohr effect, and for lineages a and g-k also DPG; $\alpha_1\beta_1$, $\alpha_1\beta_2$ contacts; IP, interior positions; Other, remaining positions. The amino acid changing NR rates calculated for these functional groups over the evolutionary lineages are smaller (probably because of the tree's low density of nucleotide sequence data) than the rates previously found in reconstructions of globin phylogeny from amino acid sequence data. Nevertheless, the trends observed for the amino acid-changing NR rates among the different functional groups and between early vertebrate and later amniote lineages generally agree with those found¹ on a dense body of amino acid sequence data. In addition, the present analysis explores a new parameter, comparison of silent NR rates to amino acid-changing ones. It also compares these rates between unexpressed pseudogene lineages and expressed gene lineages. Total NRs, the total numbers of amino acid-changing and silent NRs over all codon positions for each group of evolutionary lineages, rather than nucleotide replacement rates.

changing base replacements on this early primate γ line, not just the three at DPG-binding sites, also helped to perfect this adaptation for fetal life, is indicated by the very slow rate of evolution of catarrhine γ chains.

Complimentary evidence that natural selection directed the evolution of primate γ genes is provided by the R_{cs} values of ϵ and γ lineages of the nucleotide sequence tree (Fig. 2). Many more silent replacements than amino acid-changing ones occurred in the stem to the ϵ - γ ancestral node and also in lineages descending from this node to human, goat and mouse ϵ genes and to mouse and rabbit γ genes. However, after the divergence of primate and rabbit γ lines, most base replacements on the primate γ line were amino acid-changing ones rather than silent. This supports the inference that positive selection acted at the DPG-binding sites and many other moderating amino acid positions of the pre-catarrhine γ chains to fix adaptive amino acid substitutions.

An earlier part of this work has been reported elsewhere¹⁵.

We thank Drs N. J. Proudfoot, P. Leder, C. A. Hutchinson III, S. J. Phillips, A. Hill, S. C. Hardies and M. Edgell for supplying us with nucleotide sequence data before publication. This work was supported by NSF grant DEB 78-10717 (M.G.) and USPHS grant GM 24681 (R.E.T.).

Received 17 February; accepted 28 April 1982.

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MATTERS ARISING

Monkeys are insensitive to pyrogenic effects of human α -interferons

SHELLEKENS *et al.*¹ injected rhesus monkeys intramuscularly with preparations of human α -interferon (HuIFN- α) of two types. One came from human blood leukocytes and had a specific activity of $10^{6.2}$ U per mg protein; the other species (IFN- α_2) was derived from *Escherichia coli* by recombinant DNA techniques and had a specific activity of $10^{7.6}$ U per mg protein. Equivalent numbers of antiviral units of the two preparations gave equal protection to the monkeys against infection of the skin with vaccinia virus. Three hours after the first injection, the monkeys given leukocyte interferon (500,000 U per kg body weight) had significantly more fever and lower white blood cell counts than those dosed with the same amount of the bacterial product. It was suggested that the leukocyte interferon preparation (presumably a mixture of different α -interferon species^{2,3}) might be more toxic because it contained one or more interferon species other than IFN- α_2 . If, then, the α_2 species also produces significantly fewer side effects in man at the same antiviral dose, substantially larger amounts might be used clinically: the authors suggested the possibility of a 10 times larger dose.

Schellekens *et al.* put forward a second explanation for their results—that the toxic effects reflected impurities present in their leukocyte preparation (which was ~1% pure). Results with more highly purified HuIFN- α preparations derived in our laboratory from lymphoblastoid cells (Namalwa line) support this view. In a toxicological study, patas monkeys (4–7 kg body weight) were dosed daily for 15 days with 2×10^6 IU per kg body weight (four times more than was used by Schellekens *et al.*). The batch used had a specific activity (before addition of human serum albumin, 1.5 mg per ml, as a stabilizer) of $10^{7.2}$ IU per mg protein. There were no consistent changes in either rectal temperature at 2, 4 or 6 h after the first dose or peripheral white blood cell counts at 3, 7, 10 and 15 days. In another study (H. Nakatani, personal communication), rhesus monkeys (1.2–2.1 kg body weight) were dosed each day for 3 months with 1×10^6 IU per kg body weight of another batch with a specific activity of $10^{7.9}$ IU per mg protein. There was no significant change in rectal temperature 2 or 6 h after the first dose, or in white cell counts at any time during the

study. (No major pathological or other changes were observed in the monkeys in either study.) Both Namalwa interferon preparations, as well as other routine preparations issued for clinical use with a specific activity of $\geq 10^{8.0}$ IU per mg protein, have nevertheless produced fever and leukopenia in patients (ref. 4 and T. J. Priestman, personal communication). These were not caused by contamination with bacterial endotoxin.

It is clear that tests in monkeys cannot yet be used to predict whether a particular human IFN- α preparation will be pyrogenic in man, and phase I (dose-tolerance) studies such as those already carried out with our Namalwa interferon⁴ will be needed for each type of preparation.

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WEISSMANN AND SHELLEKENS
REPLY—Finter and his colleagues conclude from their experiments that the pyrexia arising in rhesus monkeys following injection of <1% pure, blood leukocyte interferon is due to impurities in the preparation (our second explanation¹), rather than to a particular species of interferon other than IFN- α_2 which might be present in such leukocyte interferon preparations. This conclusion is not warranted, however, because it is based on their finding that highly purified lymphoblastoid (Namalwa) interferon does not cause fever in patas and rhesus monkeys and there is no evidence that lymphoblastoid interferon contains the same spectrum of interferon species as leukocyte interferon. Thus, the formal possibility of a peculiar interferon species in blood leukocyte interferon preparations still remains.

Nevertheless, we also believe it more likely that the presence of impurities in the leukocyte interferon preparation was responsible for pyrexia in rhesus monkeys, and we agree that in regard to monitoring side effects such as pyrexia,

rhesus monkeys may not be a reliable model system.

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Do antidepressants induce dopamine autoreceptor subsensitivity?

ACCORDING to Chiodo and Antelman¹, repeated daily treatment of rats with tricyclic antidepressants results in a 'subsensitivity' of dopamine autoreceptors. Using extracellular single unit recording methods, they measured the responsiveness of neurones located within the substantia nigra zona compacta, to a low (4 μ g per kg, intravenously) dose of apomorphine. In these experiments, the effects of apomorphine were dramatically attenuated in rats that had been treated for 10 days with amitriptyline, imipramine or iprindole as compared with saline-treated rats.

We have attempted to reproduce these results by examining the effects of cumulative intravenous doses of apomorphine, using very similar electrophysiological methods. Male Wistar rats were treated twice daily for 10 days with imipramine (10 mg per kg, $n = 20$), amitriptyline (10 mg per kg, $n = 11$), or saline ($n = 35$) intraperitoneally. At 48 (± 4) h after the last treatment, rats were anaesthetized with chloral hydrate and firing rate was recorded in zona compacta neurones that had been identified according to the criteria of Bunney *et al.*². Apomorphine was administered sequentially at 90-s intervals at doses from 4 to 128 μ g per kg. As compared with saline, neither amitriptyline nor imipramine appreciably altered the inhibitory effects of any dose of apomorphine on firing rate.

Several considerations may account for our failure to duplicate the results of Chiodo and Antelman: for example, we used Wistar rats, whereas Chiodo and Antelman used Sprague-Dawley rats. This explanation seems unlikely because a second laboratory (MacNeil and Gower, see below), using Sprague-Dawley rats,

also failed to detect an antidepressant-induced reduction in nigral neuronal sensitivity to apomorphine. Another possibility is that the population of neurones sampled by Chiodo and Antelman differed from those in our experiments despite the similarity of the respective selection criteria. For example, in experiments by ourselves and others³, a 4 µg per kg dose of apomorphine caused approximately one-half the degree of inhibition reported by Chiodo and Antelman.

Evidently, some of the methodological variables essential to the neurophysiological demonstration of antidepressant-induced nigral neuronal subsensitivity have not yet been characterized fully. Until these variables are better understood, we suggest that this phenomenon be regarded as a provocative finding awaiting confirmation.

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CHRONIC treatment with an antidepressant has been reported¹ to induce a subsensitivity of presynaptic dopamine receptors. Chiodo and Antelman observed that the low-dose, apomorphine-induced reduction in the firing rate of dopaminergic cells located in the substantia nigra zona compacta was attenuated by chronic treatment with imipramine, amitriptyline or iprindole.

We have attempted to reproduce the subsensitivity effect. Groups of rats were treated chronically with imipramine or saline using the same 2-day or 10-day treatment conditions as Chiodo and Antelman. Sprague-Dawley rats (Charles River) were housed at two per cage and handled daily during the chronic treatment period. In preparation for recording from dopaminergic cells, the rats were anaesthetized with chloral hydrate (400 mg per kg, intraperitoneally) and the femoral vein was cannulated for drug administration.

Although the animals treated with imipramine lost weight and became more irritable, there was no significant difference in the response of nigral dopaminergic cells to the low-dose apomorphine challenge between the treated and the control groups. In addition, we did not obtain the magnitude of response to apomorphine in control rats that Chiodo and Antelman reported; they

obtained a reduction of ~70% in firing rate with 4 µg per kg apomorphine, whereas we found a reduction of ~35%. Our control response agrees more with the results of Skirboll, Grace and Bunney².

The subsensitivity phenomenon reported by Chiodo and Antelman seems to be elusive and may not be a general effect, but rather may be related to some unidentified aspect of their technique. This is supported by the failure of at least one other laboratory to replicate their finding (Welch *et al.*, see above).

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CHIODO AND ANTELMAN REPLY—Welch *et al.* and MacNeil and Gower report that they were unable to replicate our findings of dopamine autoreceptor subsensitivity after repeated tricyclic antidepressant treatment¹ and state that our results are 'elusive' and 'provocative' at best. Quite the contrary! Our results are part of a growing body of diverse evidence supporting the notion that repeated antidepressants do indeed induce dopamine autoreceptor subsensitivity. This evidence can be divided into behavioural, neurochemical and neurophysiological categories.

(1) Behavioural evidence: As stimulation of dopamine autoreceptors with low doses of apomorphine decreases locomotion, reduction of this hypokinesia by treatments which do not compete for the same receptor is thought to reflect autoreceptor subsensitivity. Tricyclic antidepressants^{2,3}, mianserin², monoamine oxidase (MAO) inhibitors⁴, rapid-eye-movement sleep deprivation⁴, electroconvulsive shock (ECS)⁵ and lithium⁶, all significantly reduce apomorphine-induced hypokinesia after repeated treatment. These data indicate that almost all antidepressant treatments can induce subsensitivity of dopamine autoreceptors. Neither Welch *et al.* nor MacNeil and Gower cite any of these findings.

(2) Neurochemical evidence: Two types of biochemical data support the notion of antidepressant-induced dopamine autoreceptor subsensitivity. First, repeated administration of tricyclic antidepressants^{2,11} and atypical antidepressants² decrease the ability of autoreceptor-specific doses of apomorphine to reduce dopamine metabolism. Second, Lee and Tang⁷ have recently demonstrated that chronic treatment with desmethylimipramine or nomifensine

decreased H³-dopamine binding in the striatum. As H³-dopamine is thought to bind preferentially to presynaptic dopamine receptors, these data indicate that antidepressants induce a subsensitivity of the autoreceptors located on both dopaminergic terminals and cell bodies. In addition, Koide and Matsushita⁸ have reported a reduction in striatal H³-spiperone binding after repeated tricyclic administration, suggesting that antidepressants can also induce a subsensitivity of at least some postsynaptic dopamine receptors.

(3) Neurophysiological evidence: Neurophysiological support for dopamine autoreceptor subsensitivity following repeated antidepressant treatments is also of two types. In addition to our finding that tricyclic antidepressants and iprindole can induce such subsensitivity¹, we have obtained similar results after ECS⁹ and with the MAO inhibitor, phenelzine³. The phenelzine study used a decrease in the inhibitory effects of microiontophoretically applied dopamine rather than a decrease in the effects of intravenously administered apomorphine to index dopamine autoreceptor sensitivity. Interestingly, subsensitivity after phenelzine was evident even in response to dopamine ejection (4-10 nA) which inhibited dopaminergic neuronal discharge by only 20-40%—the same degree of inhibition reported by Welch *et al.* and MacNeil and Gower after apomorphine. These data obviate the argument of these investigators that they may have been recording from a different population of neurones which were only mildly inhibited by apomorphine administration relative to those sampled in our studies. Moreover, Groves *et al.*¹² (using a treatment paradigm identical to ours) have reported that repeated ECS induces a subsensitivity of dopamine autoreceptors as shown by significant reduction in the inhibitory effects of low doses of apomorphine on dopamine cell firing. A second line of neurophysiological evidence supporting dopamine autoreceptor subsensitivity following antidepressant treatments is provided by the work of Mereu *et al.*¹⁰ showing that the electroencephalograph synchronization which results from autoreceptor doses of apomorphine is eliminated by repeated ECS.

We have now cited at least 11 studies from 5 independent laboratories which we believe strongly support the idea that repeated treatment with antidepressants reduces the sensitivity of dopamine autoreceptors. In view of this extensive support we find no basis for the statement of MacNeil and Gower that "the subsensitivity phenomenon reported by Chiodo and Antelman appears to be elusive and may not be a general effect...". The paucity of procedural detail and the absence of their actual data make it virtually impossible to guess why Welch *et al.* and MacNeil and Gower were unable

to replicate our findings. Nevertheless, it is worth noting that although tricyclic antidepressants are ineffective in some 30–40% of depressed patients, this does not obviate their usefulness in the remaining 60–70%. Finally, although these investigators failed to obtain evidence of dopamine autoreceptor subsensitivity after 10 days of antidepressant treatment (as we did), it is conceivable that autoreceptor subsensitivity developed at a later time. For example, although Lee and Tang⁷ did not observe a reduction of H³-dopamine binding in the striatum with 10 days of desmethylimipramine treatment, they did so after 28 days. In fact, given the differences in rearing conditions (for example, number of animals per cage, food supply, noise level), strains of animals used and animal suppliers between one laboratory and another, it would indeed be surprising if these two groups had observed autoreceptor subsensitivity exactly when we did.

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application of stone tools to the bones. Because of the importance of situation on the determination of Great Plains butchering strategies⁵, detailed comparison of the two cases will prove complicated, but should provide considerable benefits. Here I pursue a line of inference introduced but not elaborated on by Bunn¹, and not considered by Potts and Shipman²: the likelihood that some slicing marks resulted from skinning operations.

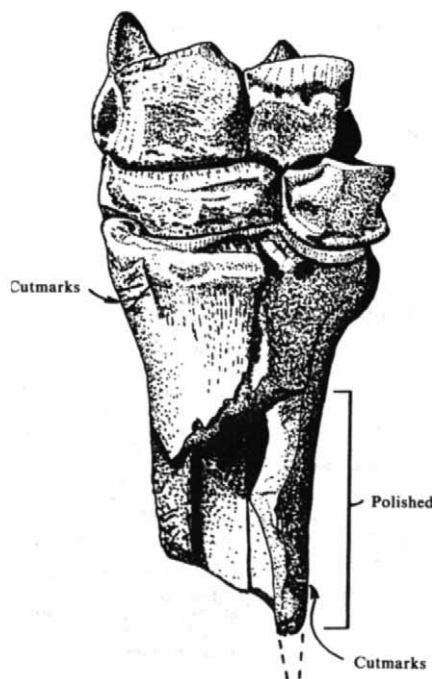


Fig. 1 Anterior view of articulated left carpal/metacarpal group, *Bison bison*, from EcPn-2, a late Holocene archaeological site in Alberta, Canada⁶. The specimen exhibits three different classes of evidence relating to butchering and cultural use as a tool: two groups of transverse cutmarks from skinning; midshaft smashing, probably for marrow recovery; and distal polish from use as a chopping or digging tool. (Drawn by author.)

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Cut marks and early hominids: evidence for skinning

BUTCHERING marks on Plio-Pleistocene mammal bones from Olduvai and Koobi Fora in East Africa have been described by Bunn¹ and Potts and Shipman². Their close similarity with cut marks from North American Great Plains archaeological sites^{3–5} provides strong support for the hypothesis that the marks resulted from

torial butchering processes prevalent in modern European and American cultures. Areas of muscle attachment can be smashed free of surrounding bone to serve as handles for leverage of the muscles. A procedure of this sort requires prior removal of the skin over a large area. Initiation of a cut or tear in the skin is one of the more difficult tasks faced by a butcher using stone tools, because matted hair, mixed with blood and fat, fouls cutting edges. However, this is greatly facilitated if the first cutting is done against a non-meat-bearing bone, so that the tool is pushed against a solid mass rather than against soft flesh. A transverse cut takes advantage of a convex skin and bone surface, to minimize fouling of the blade. Once begun, the skin tear can be extended easily into fleshy areas by means of cutting motions directed outwards, most conveniently with a hooked flake.

Use of such a skinning procedure by early hominids, as with later hunters, would have left a signature of transverse slicing marks at the point of the initial cut, on a non-meat-bearing element. Subsequent skinning operations would have left few or no marks on bones. Slicing marks are frequently seen on *Bison bison* metapodials (metacarpals and metatarsals) from North American Great Plains archaeological sites (Fig. 1), and comparable marks are less frequent on meaty upper limb bones. These cutmarks on the metapodials are typically interpreted as skinning marks^{3,5,6}. Archaeologists working with samples of deer bones from the eastern United States⁷ and llama bones from Peru⁸ have reached similar conclusions. The same hypothesis is submitted here for the metapodials from Olduvai that display such marks.

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Potts and Shipman² note that nearly half of their observed stone tool cutmarks occur on non-meat-bearing bones, whereas tooth marks from carnivores and rodents are mostly on meat-bearing elements. This suggests differing exploitative goals, and I hypothesize that a second factor beyond the need for meat, that is, the systematic removal of animal skins, operated in the case of hominids.

Stone tool cut marks on meat-bearing bones document unbuffered contact with tools. Direct contact of tools with unbroken bone shafts to leave slicing marks implies, therefore, that meat was stripped from the bones, and not simply that an animal was being quartered. Frison³ has described how individual muscles can be stripped from carcasses, a very different procedure from the sec-

Graphite genesis in high-grade metamorphic rocks

IN support of his model for the genesis of abiotic graphite by the introduction of CO₂ during deep crustal (granulite facies) metamorphism, Glassley¹ cites West Greenland² and southern Norway³ as two

areas in which "the low-grade (*sic*), hornblende-bearing material does not contain significant quantities of organic compounds, although graphite is found in higher-grade metamorphic equivalents". In fact, the comparison is between rocks of the amphibolite facies and their presumed granulite facies equivalents: there are no contemporary lower-grade assemblages in either area^{2,3}. We agree with Glassley¹ that such field evidence would indeed suggest abiotic formation of graphite in the granulite facies. However, in this context, it can be demonstrated that the reference to southern Norway (and arguably, West Greenland) is invalid.

In Norway, Glassley's example³ is an area which covers the amphibolite-granulite transition zone in the Skagerrak coastal regions of the Bamble sector, a terrain in which we have pursued field-based studies for more than a decade. Here, CO₂ was undoubtedly influential during the formation of the granulites⁴. Nevertheless, Andreae³ states clearly that "most occurrences of macroscopically graphite-bearing rocks are found between the muscovite (out)⁵ and the hypersthene⁵⁻⁷ isograds", within the amphibolite facies zone. There is also a published map⁸ which demonstrates that the major belt of biotite-garnet-sillimanite-graphite bearing supracrustals lies some 10 km outside the granulite facies zone. Equivalent graphite-bearing rocks outcrop throughout most of the Bamble sector, in both the amphibolite facies⁹⁻¹⁴ and granulite facies¹⁵⁻¹⁷ zones. The same is true for the only other graphite-bearing lithologies, the marbles and calc-silicate rocks^{3,8,18}. It is because these lithologies are not distributed uniformly throughout the complex that there are only sporadic occurrences of graphite in some sub-areas, for instance in the "medium grade [amphibolite facies] area west of Arendal"³. The more important point is that the graphite genesis is not related, in any way, to the grade of metamorphism. Consequently, this terrain does *not* provide the "unequivocal evidence for an abiotic origin" that Glassley claims¹.

We have no first-hand knowledge of the Nagssugtoqidian terrain in West Greenland, but we are familiar with the study² cited by Glassley¹. From this, it seems that the only possible comparison which might be consistent with Glassley's interpretation would be that between the graphitic, pre-Nagssugtoqidian, rocks in the granulite facies at Agto (group b), and the almost graphite-free rocks in the amphibolite facies at Egedesminde and Jacobshavn. This would be a valid comparison only if the two groups were proven equivalents, which they are not: Escher *et al.* refer to the possible correlation as 'speculative'². Interestingly, the other recognizably pre-Nagssugtoqidian group (group a) is graphite-free, yet appears to be in the granulite facies (ref.

2, Fig. 90). On the basis of this information, we submit also that West Greenland, like southern Norway, should not be regarded as providing evidence in support of Glassley's model¹.

Our studies in Norway are currently supported by NERC grant no. GR/3773 (D.F.) and Queen Mary College, London (I.C.S.).

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GLASSLEY REPLIES—The graphite genesis model I proposed¹ is a consequence of the physicochemical behaviour of C-O-H fluids in equilibrium with a hydrogen modifying mineral assemblage. As the chemical conditions necessary for graphite formation can be obtained in many mineral assemblages, graphite genesis from a CO₂-rich fluid phase will occur in nature in a variety of rock types. However, documentation of this process is difficult because of the likelihood of simultaneous generation of graphite through oxidation of organic material and through the inorganic process I outlined. The evidence I cited for inorganic graphite genesis^{2,3} were instances where published results documented substantial increases in graphite abundance related to metamorphism. Field and Starmer have challenged my assertion that field observations in southern Norway and West Greenland provide evidence that the graphite genesis model was operative in these locations.

My paper emphasized the fact that organic material is virtually non-existent in hornblende-bearing material in the cited areas, while graphite is a common

minor phase in high-grade rocks which are compositional equivalents of the lower grade, hornblende-bearing rocks. Field and Starmer have incorrectly equated hornblende-bearing material with amphibolite facies rocks in general. This was neither a correlation made in the paper, nor a petrologically sound conclusion: hornblende granulites are well known^{4,5}, as are hornblende-absent amphibolite facies rocks⁶. The distinction I originally emphasized is thus a distinction based on specific assemblages, not a distinction dependent on facies classifications.

The validity of the criticism of Field and Starmer thus depends on the documented occurrence of graphite with hornblende in the particular rocks discussed and not upon whether graphite is present in amphibolite facies assemblages in general. Careful reading of the papers they refer to provides no documentation of the coexistence of hornblende and graphite. The fact that graphite is present in Norway in rocks of facies other than the granulite facies has no bearing on the argument.

Their discussion regarding the Greenland material lacks substance. The significant point remains that pre-Nagssugtoqidian rocks in which graphite is present in high-grade regions are correlated with lower grade rocks in which graphite is lacking. The 'speculative' nature of this correlation results solely from the fact that one sequence contains graphite, while the other does not, although all other lithological and structural features of the region support the correlation. Indeed, it is precisely this contrast which the inorganic graphite genesis model resolves. More recent work^{7,8} is consistent with the proposed correlation.

Graphite genesis through the mechanism I outlined requires the presence of a CO₂-rich fluid phase of the appropriate oxygen fugacity. The absence of graphite in many high-grade metamorphic terranes simply suggests that the fluid composition was such that graphite was not stable; the observation that graphite does not occur in some high-grade rocks in no way challenges the proposed model.

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BOOK REVIEWS

Snippets of science

Donald S. Fredrickson

LEXICOGRAPHERS have been slow to embrace them, but John Ziman's inventive nouns, such as *consensibility*, enjoy status among those who appreciate insightful views of the workings of the scientific community. Professor Ziman's readers will be pleased by the arrival of a new compendium of "occasional papers" from the pen of the well-known communicator of science, whose views on the subject come from a position inside it, as Director of the H.H. Wills Physics Laboratory, and from outside it, in his capacity as Chairman of the Council for Science and Society.

This new Ziman release consists of 42 essays, book reviews, BBC broadcasts and other comments written in the years between 1956 and 1979. So eclectic a pudding naturally varies in consistency, but *Puzzles* is chock-full of tasty quotes and observations. A thorough index permits selected sampling and enhances the reference value of the book.

The articles are grouped in eight sets, the largest of which deals with intellectual and social aspects. Of these papers, "Science is Social" (1960), and "Is Science to be Believed?" (1979), represent, respectively, the conceptual birthplaces of *Public Knowledge* (Cambridge University Press, 1968) and *Reliable Knowledge* (Cambridge University Press, 1979), the books by which Professor Ziman is best known to the public and most scientists. None of the short pieces here provides the satisfaction of a thesis fully developed in the manner of the books. There is a different kind of pleasure to be had, however, in scanning the author's broad range of interest in science: research as an art and as a profession, science in the Third World, scientific communications and education, relations with Soviet science, and the confrontations between science and society.

In only two of the essays does Professor Ziman's control of his subject seem to falter. In "Some Pathologies of the Scientific Life" (1971), rage directed against conscious deceit by a few scientists ends, curiously, with a peevish apology for being trivial. And in "From Parameters to Portents — And Back" (1978), he flails unconvincingly at the immensely popular pastime of judging scientific productivity through manipulation of quantitative indicators. We are led deeper and deeper into a bewildering maze of output/input measures which defy comprehension. By the time Professor Ziman's "geometrical metaphor" has reached the *cognitive*

Puzzles, Problems and Enigmas: Occasional Pieces on the Human Aspects of Science. By John Ziman. Pp.373. ISBN 0-521-23659-2. (Cambridge University Press: 1982.) £12.50, \$24.95.

dimension, one imagines panels of science advisors groping for the exits. As the essay comes to rest on the observation that "particular indicators may involve circular arguments, but the whole enterprise is not spherically senseless", we sense a talent for farce that merits cultivation.

Such rare excursions aside, John Ziman appears in this volume as a graceful and lucid reviewer, a clear thinker and a most perceptive observer of a profession to which he is intensely loyal. He is at his best in explaining the personal, intuitive and

subjective sides of science, especially how they relate to the intellectual transactions which characterize science as a "social product". The lectures on science in Third World countries reveal sensitivity and good sense. Perhaps the best piece in the collection is a recent essay, first published in 1978, on "Solidarity Within the Republic of Science". It is an appeal to scientists and their organizations to recognize that the universality of their community requires more positive action in defence of human rights and scholarly freedom. It is a form of *consensuality* most in accord with the ultimate purpose of science. □

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Scientific prince of a Victorian kingdom

Roy MacLeod

John Tyndall: Essays on a Natural Philosopher. Edited by W.H. Brock *et al.* Pp.219. ISBN 0-86027-008-4. (Royal Dublin Society, Ballsbridge, Dublin 4, Ireland: 1982.) Ir£10 plus Ir£1.50 post and package.

ASIDE from those whose experience of school physics may recall the mysteries of the Tyndall Effect, the work and influence of the eponymous successor of Faraday is virtually unknown to students of the present generation. Even fewer will recall that Tyndall was, both by birth and by political commitment, an Irishman of the Orange persuasion. Both facts receive belated but welcome recognition in this third volume of the new *Historical Studies in Irish Science and Technology*.

"Science is before long to rule the world", observed *Vanity Fair* in April 1872, "and Mr Tyndall is one of the pioneers of the kingdom". Whether as public lecturer, experimentalist, Alpinist, examiner, educator or advisor to government, Tyndall was among the best known and most cited publicists of Victorian science. Beginning with his *Heat — a Mode of Motion* in 1863, continuing with his *Fragments of Science for Unscientific People* in 1871, and with his *New Fragments* in 1893, he spoke to a traditional culture which, in his view, re-

quired a fresh and dynamic appreciation of Nature's wonder and a willingness to apply man's new knowledge of natural phenomena to the purposes of social reform. With Huxley, Hirst and Spencer, he popularized the radical research programme which became associated with the principles of "scientific naturalism", denying the inevitability of materialism, yet rejecting the fetters of religious belief that would limit the domain of scientific knowledge. By his death in 1893, Tyndall's road to agnosticism was well travelled by many who had neither read nor heard his words. With others of the famous X-Club he preached a liberalism that reached beyond party (especially beyond Gladstone after 1855); an Emersonian independence of mind that spoke directly to the working men of England; and a Carlylean hatred of sham that bore witness to the abiding power of the scientific imagination.

Tyndall's own imaginative grasp, and his range of international interests, gave him a wide, if not always a deep command of scientific work in many fields and across the world. His famous lecture-tour in America in 1872-1873 is still commemorated by research studentships created in his honour. In his scientific work personal friendships mattered greatly to him; and a profoundly happy marriage, late in life to Louisa Hamilton, has left us, through their

diaries, with a moving testament to a contented unity of life, work and affection. Their contentment was shattered when an accidental dose of chloral ended his life — and, alas, nearly his reputation too. Within two years of his death, the discovery of radioactivity proclaimed the advent of a “new physics” and Tyndall’s classic works on sound and heat, dust and light, no longer held centre stage. Even the brilliance of his popular writings was obscured by a succession of rapid movements which overtook English science at the turn of the century.

Meadows, Irena McCabe, Con O’Rourke, J.S. Rowlinson, E.J. Wiseman and Philip S. Callahan — are complemented by chapters dealing with his popular impact, by Sophie Forgan, Donald Thompson, J. Vernon Jensen, Frank M. Turner, Anna T. Cosslett, Katherine R. Sopka and Charles A. Taylor. Biographical sketches by Joe Burchfield and Norman McMillan and Martin Nevin introduce the volume, and complete the personal portrait.

To this assessment, Ronald Clark adds an engaging tribute to Tyndall as a mountaineer. This seems particularly apt. Visitors today to Bel Alp, near Brig, will find an obelisk honouring Tyndall which overlooks a glacial valley of great beauty.

To reach it requires energy, good luck and a firmity of purpose, qualities which epitomized the man who conquered the Weisshorn. The easy parallel between the measured progress of the experienced climber, and the upward progression of the young Irish railway surveyor, rising to the fashionable heights of Victorian science, does not escape Tyndall’s biographers. As Tyndall and his contemporaries knew, in mountaineering, as in science, it is for the magnificent view that one endures the hardest climb. □

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IMAGE
UNAVAILABLE
FOR
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REASONS

“Prof. Tyndall as ‘Our Literary Man’,”
from *Moonshine* August 25th, 1883.

This celebration comprises 16 illustrated essays, sketching in most useful detail those several compartments of life and knowledge in which Tyndall’s influence was felt. Together with a few standard articles and catalogues, which give the essential coordinates to Tyndall’s career (most of which, in the absence of a bibliography, are mentioned in footnotes), this collection will provide a useful Baedeker to scholars following the main roads and some of the forgotten byways of Victorian science. It will certainly serve us well until a full biography arrives to update and replace the worthy but incomplete account, *Life and Work of John Tyndall*, published by A.S. Eve and C.H. Creasey nearly 40 years ago.

Through his writings, Tyndall became known to his contemporaries not least for a long series of controversies and priority disputes, on issues ranging from the conservation of energy to spontaneous generation, from glacier motion to lighthouse illumination. In this collection, these debates — ably explained by A.J.

Centres or circuits: the hypothalamus revisited

Susan Iversen

Handbook of the Hypothalamus. Vol.3, Parts A and B, *Behavioral Studies of the Hypothalamus*. Edited by Peter J. Morgane and Jaak Panksepp. Part A pp.499, ISBN 0-8247-6094-X; part B pp.464, ISBN 0-8247-6095-8. (Marcel Dekker: 1981.) Each part SwFr.220, \$93.50.

THE *Handbook of the Hypothalamus*, the first two parts of which were reviewed in *Nature* 293, 764 (1981), is completed by the publication of these companion volumes on behavioural studies. Students of physiological psychology at the undergraduate and graduate level will find them invaluable sources of information and enlightened comment.

Two themes dominate these volumes. First, it is apparent that the hypothalamus is not merely a funnel interconnecting widely separated levels of organization in forebrain and brainstem, but contains groups of neurones with properties fundamental to the integrated function of this widespread circuitry. In consequence the current tendency to ascribe homeostatic impairments after hypothalamic damage solely to transection of neural pathways traversing the hypothalamus, rather than to damage of its intrinsic neurones, seems to me misguided.

Second, it is implied that it was the discovery of specific chemically coded pathways of the CNS which undermined the prevalent use of the concept of the “centre” to explain hypothalamic control. I am not sure that this is so. The idea that specified levels of control reside in localized areas of brain was under attack long before chemical communication in the brain was generally recognized to be of functional importance. Rather we clutched at straws, hoping that by unravelling chemical interactions in centres previously defined with lesion and stimulation techniques, we would finally understand how the hypothalamus integrates endocrine and

viscero-motor control. As Morgane notes, centres were replaced by chemical pathways and the illustration on the covers of the books portrays this metamorphosis. One assumes that in selecting such a picture the editors do not accept this form of approach as being the more enlightened of the two.

Volume 3A begins with a masterly chapter by Boulant on temperature regulation, which involves such a wide range of physiological and behavioural responses that it lends itself to neuronal modelling. Temperature regulation is again the focus of the next contribution, by Myers, who provides an excellent summary of the evidence implicating NA, DA, 5HT, ACh in such regulation. Sadly, though, in a brief section he gives neuropeptides only cursory treatment. There is now evidence that a number of peptides are involved in temperature control: bombesin, neurotensin, cholecystokinin, somatostatin (all physiologically active gut peptides, which also exist in the brain) and β -endorphin induce hypothermia, whereas TRH induces hyperthermia. It is not prophetic to predict that within a decade peptides will be shown to be as, if not more important than the classical transmitters in temperature regulation and other hypothalamic functions.

Still in Vol.3A, Powley *et al.* describe in detail the syndromes of hyperphagia and aphagia seen after bilateral ventromedial and lateral hypothalamic lesions respectively, and conclude that although there are permanent changes after lesions the animals show no absolute loss of their ability to respond to acute challenges and are able to defend the new body weight. These results strongly suggest that energy homeostasis involves substrates far removed from the hypothalamus, and that although there are primary deficits after VMN and LH lesions any formulation of the syndromes in terms of a single element

Marvell Collection

of behaviour, or of a single acute regulatory response, are likely to be inadequate.

The work of Leibowitz on the neuropharmacology of food intake is included in this volume; the *Handbook* would have been incomplete without a review of these elegant studies. The same may be said of Rolls's work on the neurophysiological responses of limbo/hypothalamic neurones in the monkey.

In the opening contribution to the second volume, water and salt regulation of body fluids is admirably dealt with by Hatton and Armstrong. A wealth of anatomical and physiological results have accrued since the appearance of Fitzsimons's classic review (*Physiol. Rev.* 52, 468-561) in 1972. Body fluid is controlled by a number of sensitive and closely inter-related physiological mechanisms at the whole-body level. Attempts have been made to understand how information about such variables is relayed to CNS, how they come to influence the hypothalamus, result in hormonal secretion and induce water- and salt-seeking behaviour. Thus far, however, little attention has been given to the role of chemical transmitters and neuropeptides in the brain in controlling water and salt intake; one would hope that this will soon be remedied.

The volumes provide much evidence of the kinds of stimuli the hypothalamus responds to. But how does hypothalamus influence autonomic, endocrine and behavioural responses? In Vol.3B Mancia and Zanchetti provide a useful chapter on connections to autonomic centres of brain stem. Later this issue is taken up somewhat tangentially by Wayner *et al.*, in a contribution which is to me one of the most thought-provoking in the volumes; it discusses the unlikely topic of adjunctive behaviour.

Rats receiving dry food on an intermittent basis consume water in huge quantities, out of all proportion to their physiological needs. Wayner and his colleagues see this behaviour as an enhancement in the readiness to respond in general, to drink in the particular, and liken it to the enhancement of these behaviours seen after water deprivation, lateral hypothalamic stimulation or infusion of hypertonic saline. The latter manipulations are shown to enhance the excitability of spinal reflexes, and it is claimed that the lateral hypothalamus has a major influence on a brain stem motor control system that determines spinal reflex excitability and motor activity. Thus the rats drink because of enhanced spinal excitability initiated by the lateral hypothalamus. Because of this change in responsiveness, the sensory stimuli associated with the act of drinking (including feedback from the muscles) are likely to activate the CNS and initiate the same act again; hence behaviour becomes repetitive and ultimately stereotyped.

Although the model focuses on Wayner's own experiments performed on the spinal cord, it may be extended to

involve forebrain motor systems. In addition to its influence on spinal cord, the hypothalamus projects to the forebrain dopamine neurones of the ventral mesencephalon, which control striatal motor function. Drugs enhancing dopamine release result in repetition of species-specific motivated behaviours, which is advantageous during learning but when uncontrolled during behavioural stereotypies disrupts normal motivated behaviour. Thus it is tempting to speculate that the hypothalamus may simultaneously enhance response probability at forebrain and spinal levels of organization.

It falls to Panksepp himself to take on the thorny problem of relating the physiological properties of the hypothalamus to the theoretical concept of motivation, the construct used to describe variations between physiological homeostasis and the behaviour of the whole organism. Animals will press a lever to obtain electrical stimulation of the hypothalamus, a site thus considered central to the mechanism of reward and learning. Furthermore, since stimulation of the same site induces species-specific acts of behaviour such as feeding and drinking, the same systems are implicated in the initiation of motivational behaviour; so assumption of an intrinsic relationship between rewarding stimuli and motivated behaviour is germane to all theories of learning.

Panksepp provides an excellent review of all these experiments and proposes that the hypothalamic substrates constitute a non-specific "approach-investigate command motor and sensory system". When activated this system increases the probability that behaviour will occur, and the nature of the incentive (or rewarding) stimuli the animal encounters determines the specific profile of behaviour observed. Thus motivation is viewed as a non-specific energizer of behaviour rather than a set of state-specific centres in the hypothalamus triggering the appropriate behaviour. Such a theory of motivation has been entertained by others in recent years. It is an attractive one and accounts for a striking number of observations on intracranial self-stimulation, brain stimulation and tail-pinch induced behaviours.

The question, however, is whether a non-specific arousal system can account for *all* the facts. Clearly, Panksepp is torn on this one and throws out the suggestion that transhypothalamic non-specific arousal systems may interact with specific hypothalamic centres controlling one aspect of species-specific behaviour rather than another. While I too have been attracted by arousal theories of motivation, I am increasingly aware of evidence for specific neural processing intrinsic to the hypothalamus; I think Panksepp is correct to bide his time on this problem. □

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Neglected reptiles

Barry Cox

Mammal-Like Reptiles and the Origin of Mammals. By T.S. Kemp. Pp.362. ISBN 0-12-404120-5. (Academic: 1982.) £24, \$49.50.

THE mammal-like reptiles always seem to have been overshadowed by their more glamorous and bizarre cousins, the dinosaurs, even though they were at least as diverse and dominant during much of their hundred-million-year existence. Indeed, the only previous scientific book on them, *The Mammal-Like Reptiles of South Africa*, published 50 years ago, was written by Robert Broom and was devoted only to the South African forms that he described and named so prolifically. Tom Kemp's book is therefore a long-overdue recognition of the importance of this first radiation of reptiles, and gives us the first coherent treatment of the evolution of the whole group and of the problems of life-style and of interpretation that they pose.

The bulk of the book is devoted to an account of the different groups of mammal-like reptile, from the Early Permian pelycosaurs of North America and Europe to the Late Permian and Triassic groups that are found throughout the world. In most cases, after describing the morphology and systematics of each group, Kemp deals with its feeding mechanism, middle ear structure (for there has been much controversy on this system in synapsids), locomotion and general biology. It was, of course, from the cynodont synapsids that mammals arose, and Kemp also provides a review of Mesozoic mammals in general and of the gradual evolutionary transition from reptile to mammal which is, as he notes, "the only such major transition in the animal kingdom that is anything like well documented by an actual fossil record". In a final chapter, Kemp comments on the extent to which this record also provides evidence on the pattern of origin and evolution of higher taxa such as families and orders.

The book is clearly written and laid out, and has a bibliography of over 200 entries that includes all of the more recent, relevant palaeontological literature. Kemp has also reproduced some of the important figures from this literature. Though many of these are clear, a few have been too greatly reduced; as a result, the more darkly shaded areas have coalesced into unrelieved darkness, while the finer lines have disappeared altogether. However, this is a minor failing in a useful and comprehensive book, which any university library will need and which will be welcome by all workers on the evolution of reptiles and the origin of mammals. □

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The Asian monsoon as a geophysical problem

Robert M. Chervin

Monsoon Dynamics. Edited by James Lighthill and R.P. Pearce. Pp.735. ISBN 0-521-22497-7. (Cambridge University Press: 1981.) £50, \$130.

TOWARDS the end of 1977, a large number of representatives from the different geophysical sub-disciplines gathered in New Delhi with the aim of assessing the (then) current state of understanding of the processes involved in the monsoon cycle. An equally important purpose was to provide guidance and encouragement to those members of the community with the operational responsibility for local weather and flood forecasting. Indeed, the full title of the symposium — "Monsoon Dynamics, with Applications to Weather Forecasting and Flood Prediction" — gave emphasis to this intended dual function of the meeting.

This volume, which contains edited versions of 46 of the 53 research papers presented at the meeting, is a fairly complete record of the symposium. The individual papers are placed in one of five sections, primarily according to the time and space scales of the particular phenomena being investigated, or to the general approach involved. Each section is preceded by two to three pages of introductory material by one of the editors which preview the subsequent papers and place their contributions within the context of the larger multi-disciplinary objects of the symposium.

In Delhi (and thus in the book) the observational papers ran a gamut from the rather straightforward descriptive presentation of the geographical distribution of rainfall patterns to some sophisticated diagnostic analyses of atmospheric energetics and wave interactions. Data culled from a variety of different observing systems — among them aircraft, ships, super-pressure balloons and satellites — were employed. A few papers reported the results of the MONSOON-77 experiment, a precursor to the larger scale Monsoon Experiment (MONEX) which took place in 1979 as part of the Global Atmospheric Research Program (GARP). A particularly noteworthy paper was that by J.C. Sadler and J.T. Lim, which demonstrated the value of geosynchronous satellite data for determining upper troposphere level winds in the tropics — information not easily obtained from conventional observing systems.

The modelling and theoretical papers explored various interactions between the different components of the geophysical system. The models, both numerical and laboratory types of varying complexity, have been used for short-range weather and storm-surge prediction experiments, atmosphere and ocean circulation simu-

lations, and climate sensitivity studies. Experiments in this latter category are particularly useful in assessing the impact of altered surface conditions on the dynamic and thermodynamic aspects of the monsoon. The relevance of such experiments to the real world, is of course, highly dependent on the degree to which the basic model is able to reproduce the observed climate. Notable among the modelling papers, therefore, is the intriguing contribution by the late J.G. Charney and J. Shukla. This indicates that climate prediction for the tropics may be possible with atmospheric general circulation models if proper account is taken of the variability of such boundary interfacial properties as ocean surface temperature, albedo and soil moisture.

The production quality of this volume (especially of the graphical reproductions) is for the most part superb, though a smattering of typographical errors does

reduce the level below perfection. More varied is the quality of the papers themselves. Many have the appearance of being cut and pasted from other articles by the authors, and obviously they do not represent original contributions. However, the most serious criticism that may be levelled against the book is that it has appeared almost four years after the symposium took place. This lack of timeliness is made all the more apparent by our recent, enhanced appreciation of the complexities of the Asian monsoon which has resulted from the preliminary analysis of data from MONEX.

Nevertheless, within the given historical perspective, the symposium did serve an important function in focusing attention upon monsoon studies, and this volume will serve as a useful reference for those with a newly acquired interest in the Asian monsoon as a geophysical problem. □

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A fresh look at New World monkeys

Alan Dixon

Ecology and Behavior of Neotropical Primates, Vol. 1. Edited by Ademar F. Coimbra-Filho and Russell A. Mittermeier. Pp.496. (Brazilian Academy of Sciences: 1981.) \$25. Available from Dr R.A. Mittermeier, Dept Anatomical Sciences, Health Sciences Center, SUNY, Stony Brook, NY 11794.

STUDIES of the ecology and behaviour of New World primates are gathering momentum after a long period of neglect in favour of research on Old World monkeys and apes. This book, the first of two companion volumes, provides a timely and useful review of recent work. As the editors suggest, it should appeal particularly to students who are "interested in Neotropical monkeys, but do not have access to much of the published material".

The first section of the book is devoted to systematics and Mittermeier and Coimbra-Filho include a well-illustrated guide to the numerous species and subspecies of New World monkeys. Rosenberger deals with the higher taxa and his treatment of the historical aspects is thorough and scholarly. However, he proposes a major revision of the group whereby the marmosets and tamarins (usually placed in a distinct family, the Callitrichidae) are united with *Saimiri*, *Cebus* and *Callimico* in the family Cebidae, whilst all other forms are placed in the Atelidae. Many workers may judge this arrangement to be confusing and of questionable validity. The origin of the

New World monkeys also remains a controversial topic. Thus, as Rose and Fleagle point out, the fossil record is insufficient to decide whether their ancestors originated in North America or "rafted" across from Africa during the late Eocene.

Most of the book consists of chapters on individual genera and there are profiles for *Callimico*, *Aotus*, *Callicebus*, *Saimiri*, *Pithecia*, *Chiropotes* and *Cacajao*. Very little is known about some of these monkeys, such as the sakis and uakaris, but whenever possible the authors have included previously unpublished material in their reviews. One weakness is that little attempt is made to relate the physiological aspects of reproduction to the accounts of reproductive behaviour or seasonality. This bias in favour of field studies and observations of behaviour under natural conditions is understandable at this stage, however, particularly because many of these monkeys are endangered because of destruction of their rainforest habitat.

The second volume, still in preparation, will deal with the problem of conservation and will include detailed accounts of the remaining genera. Together, the two books should represent a valuable contribution to the literature on New World primates: I hope that libraries, research workers and students will buy them. □

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Minding your language in the 1980s

John Maddox

A Supplement to the Oxford English Dictionary Vol. III, O-Scz. Edited by R.W. Burchfield. Pp.1,596. ISBN 0-19-861124-2. (Oxford University Press: 1982.) £55. To be published in the United States in October.

THE *Oxford English Dictionary* (OED) is less a book than a way of life. The first and only complete edition of the dictionary took more than half a century to complete (in 1933). Since 1957, the lexicographers at Oxford have been labouring at what they call the *Supplement*, the third volume (out of four) of which, covering the entries O to Scz, has now appeared. The next and final volume is advertised for 1985, by which time no doubt some committee will have decided whether there should be a supplement to the *Supplement*.

The guiding principle has been to record and analyse the ways in which people choose to use language, not to tell them how they should (c.f. Webster). The *Supplement*, then, is a celebration of the richness rather than the purity of English.

This volume includes a long discussion of the derivation and usage of "OK" as an adjective, noun and verb (as in "to OK"). The use of the noun *perk* in the sense of an extra benefit is promoted from *slang* to *colloq.* Occasionally, however, there are stern editorial notes that "the over-use of this word in various loose senses has attracted frequent hostile comment" — e.g. *scenario*. The spirit, however, is catholic. The result is that the book is a way of finding what other people mean when they write in English.

The editor of the *Supplement*, R. W. Burchfield, and his contributors have been saying since the project began that a large part of their task would be to come to grips with what science has lately done for the English language, yet on the face of things that influence may be smaller than had been thought. True, this volume of the *Supplement* is packed with definitions (*phage*, *oncogene*, *schistosoma* etc.) whose need would not have been apparent half a century ago, but scientific words do seem to have had only a small influence on the language generally.

The words beginning with *onco* seem still to be largely technical (and words such as *onco-hazard* have not yet been coined), while the chief new sources of richness in the language seem to be malleable and simple words such as *on* (as in *on-line*) and *put* (as in *putting it across*, which H. L. Mencken insisted refers to the throwing of a baseball accurately above the "plate").

With that said, the *Supplement* has done well by its scientific words, chiefly by its succinctness. Thus *quantum* in physics is defined as "a minimum amount of a physical quantity which can exist and by multiples of which changes in the quantity occur". *Polywater* is put firmly in its place

with the phrase "a supposed polymeric form of water . . .". The definition of *quasar* as "any of a class of celestial objects that give a star-like (i.e. unresolved) image on a photograph and have a large red-shift usually taken to indicate great remoteness and immense power" is forgivably fuzzy and (now) incorrect, but the definition of *quark* is nicely enlivened by a personal communication from Murray Gell-Mann explaining that he had used the pronunciation "quork" until he recognized that he had subliminally taken over the word from *Finnegan's Wake*.

The net that has caught this range of scientific words has plainly had a fine mesh. *Poly-some* is included, as are *poly-A*, *podzol*, *planetismal*, *plasmid* and *pleiochroic*. The treatment of proper names is splendidly thorough — Poisson's distribution and brackets are both defined, as is Planck's Law, but the *Supplement* may have drawn the definition of *Planckian* too narrowly as "of, pertaining to or being a black-body".

The OED is uniquely a data base, as the saying goes, which will suggest to many that it should by now be stored on a long reel of magnetic tape, floppy discs or some other means of data storage. So it is mystifying that the publishers still find it possible to organize such a huge project while relying on traditional hot metal techniques for setting the type. Perhaps they are calculating that by 1985, when the *Supplement* (barring accidents) should be complete and when the natural question arises whether the OED as a whole should be re-published, techniques of optical character recognition will have reached the point at which a computer could take over from human typesetters.

Re-publication will however be more than a merely mechanical operation. The *Supplement* is replete with amendments of entries in the original edition such as "Delete 'Now somewhat archaic' and substitute 'Now restored to general use'" (for the adjective *obscene* used in the sense of "offensive" rather than "lewd"), or with "for 'a man' read 'a person'" as in *picketer*. It is however to be hoped that Oxford will tackle the job, and that the result will include a printed version of an enlarged OED. For the pleasure and much of the value of this huge enterprise lies in the way in which neighbouring and loosely related entries illuminate each other.

Meanwhile, one minor puzzle remains to be cleared up. Entries in this volume of the *Supplement* finish with Scz (the last entry is *Scythism*) but the next volume is to begin with Se. Is it to be inferred that nothing has happened in the past quarter of a century to such delectable words as *'Sdeath* and *sdeign*? □

John Maddox is Editor of Nature.

NEW

FORAMINIFERA

J. R. HAYNES

Reader in Geology

University College of Wales

December 1981; £50.00;
480pp; 34pp plates; ISBN 0
333 28681 2

CONTENTS

Preface; The Scope of Foraminiferal Studies; Collection, Preparation and Examination (including Annotated Literature on Technical Methods); The Living Foraminifer; Test Morphology and Composition; Classification of the Foraminifera; The Agglutinating Foraminifera; The Fusulinida — The Miliolida; The Nodosariida; The Buliminida; The Robertinida; The Rotaliida (Smaller); The Rotaliida (Larger); The Globigerinida; References; Index.

This work is an advanced text covering the biology, morphology and classification of the Foraminifera and their use in stratigraphy and palaeoecology.

Superbly illustrated, the text draws on the author's experience in oil exploration and twenty years' teaching at both undergraduate and postgraduate level.

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Mathematics

BIEDENHARN, L.C. and LOUCK, J.D. The Racah-Wigner Algebra in Quantum Theory. Encyclopedia of Mathematics and its Applications, Vol. 9. Pp.534. ISBN 0-201-13508-6. (Addison-Wesley: 1981.) \$54.50.

HOLMES, M.H. and RUBENFELD, L.A. (eds). Mathematical Modeling of the Hearing Process. Lecture Notes in Biomathematics, 43. Proceedings of the NSF-CBMS Regional Conference, July 21-25 1980, Troy, NY. Pp.104. Pbk ISBN 3-540-11155-7/0-387-11155-7. (Springer-Verlag: 1981.) DM 19.50, \$9.10.

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